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Identification of Horned and Polled *Bos taurus* using a Gene Test

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A Dissertation

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Amy Chamberlain

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science (Honours).

Identification of a Horned and Polled *Bos taurus* using a Gene Test

By

Amy Chamberlain

The absence of horns in *Bos taurus* is under the genetic control of the autosomal dominant *Polled* locus which has been genetically mapped to the centromeric region of cattle chromosome 1 (BTA1). The position of the *Polled* locus on BTA1 has been identified and candidate causal mutations have been identified. Recently it was demonstrated that there are at least two different alleles at the *Polled* locus in cattle. For example, a 202-base pair (bp) insertion-deletion (InDel), referred to as P_{202}ID, has been identified in various cattle breeds, including cattle in Scandinavia, Scotland, England, the Channel Islands and France (regions North of the Alpine region). Little is known about breed to breed variation, other than the identification of a Holstein *Polled* mutation occurring at 932 kb, and that this overlaps with a Simmental *Polled* locus at 212 kb.

Using a polymerase chain reaction – single strand conformational polymorphism (PCR-SSCP) approach, 876 New Zealand (NZ) cattle from five British cattle breeds (South Devon, Belgian Blue, Hereford, Shorthorn and Holstein-Friesian) were sampled, of which 467 were investigated to validate a polled/horned gene test developed by the Lincoln University Gene Marker laboratory. Three PCR-SSCP banding-patterns were identified, and these were typically found in homozygous polled (BB), heterozygous polled (AB) and homozygous horned (AA) cattle. Phenotypic data for polled/horned was available for all 467 cattle, so the accuracy of the gene test was calculated for each breed studied. A high level of confidence can be held when using the gene test in breeds such as the South Devon (100%), Shorthorn (100%) and Belgian Blue (96.5%), but a lower accuracy was observed with Hereford cattle (73.5%). The Holstein-Friesian cattle that were typed were true to phenotype (100%), but the result is less reliable as the sample size was small and no polled Holstein-Friesians were typed.

Overall these results suggest that the gene test has the ability to identify polled and horned genotypes in South Devon, Belgian Blue and Shorthorn cattle, but it requires further refinement for Hereford and Holstein-Friesian.

Keywords: Polled, horned, *Polled* locus, phenotype, genotype, homozygous, heterozygous, Hereford, South Devon, Belgian blue, Shorthorn, Holstein-Friesian.

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Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

Currently, the world cattle population is estimated to be about 1.3 billion head (Brown, 2009) of which a large proportion are horned. Historical records indicate the presence of naturally polled cattle in ancient Egypt (Allais-Bonnet *et al*., 2013; Roman, 2004), and until recently horned cattle were desirable, because it simplified their tethering and attachment to harnesses.

In modern day husbandry systems, such practises are no longer used and the presence of horns increase the risk of injury to both animals and handlers, especially as the housing densities in feedlots and stocking rates have increased. The presence of horns induces economic losses in the cattle industries due to dehorning practises and the treatment of subsequent secondary infections, but also due to carcass and leather deterioration from injuries (Allais-Bonnet *et al*., 2013; Medugorac *et al., 2012;* Prayaga, 2007). Since all dehorning methods are invasive and raise animal welfare issues, the ability to breed genetically polled cattle is an obvious alternative.

Since 1906, the polled phenotype has been known to be inherited as an autosomal dominant trait (Spillman, 1906) and the *Polled* locus was mapped to bovine chromosome 1 (BTA1 for *Bos taurus)* in 1993 (Barendse *et al*., 1993). In past years, the position of the *Polled* locus on BTA1 was refined (Drogemuller *et al*., 2005; Seichter *et al.,* 2012) and candidate causal mutations were identified (Allais-Bonnet *et al.,* 2013; Medugoric *et al.,* 2012; Glatzer *et al.,* 2013). Recently Medugorac *et al.,* (2012) demonstrated the existence of at least two different alleles at the *Polled* locus in cattle. For example, a complex 202-bp InDel, referred to as P_{202}ID, has been identified in various cattle breeds, including cattle from Scandinavia, Scotland, England, the Channel Islands and France (North of the Alpine regions).

Past research has enabled the development of Gene marker tests and gene tests such as the Australian Gene Poll test to successfully identify genotypes across a vast selection of breeds. However, accuracies have varied between breeds, with focus driven towards common breeds in Australia. There is therefore room in New Zealand (NZ) for the commercialisation

of a gene test that can identify the polled genotype, reducing the time and cost of removing horns from the beef and dairy herds in NZ.

Chapter 2

Literature Review

The Beef and Dairy industries are economically valuable for New Zealand (NZ), bringing large amounts of income into the country. NZ accounts for over a third of the world's dairy trade and in 2016 it contributed 29% of NZ's merchandise export earnings (Ballingall & Pambudi, 2017). Dairy is the largest goods export sector, averaging $14.4 billion of export revenue over the last five years (Ballingall & Pambudi, 2017), and averaging 7.2% per year, for the past 26 years (Ballingall & Pambudi, 2017).

Beef is one of the most commonly exported meats from NZ, with 200,000 tonnes exported to the USA in 2015/2016 and a further 70,000 tonnes exported to China (Beef and Lamb NZ, 2016). In the year ending June 2015, meat and meat-related products exports were worth NZ$6.8 billion to the economy (NZ Trade and Enterprise, 2016)

NZ is very efficient at producing dairy and red meat products, but it is a niche player in world markets. The industries have excellent food safety records and it is considered that NZ leads the world in improving food processing and marketing, while also maintaining high animal health and animal welfare standards.

Livestock production in NZ is constantly being reviewed and studied to further develop the beef and dairy industries and to meet ever changing demands. Among the approaches used, the use of genetics and breeding is one means of improving both production efficiency and product quality. Of all the traits that might be considered for improvement, the value of having horned cattle in the modern beef and dairy production systems is now being questioned. While horns are common in many beef and dairy cattle breeds, they pose animal welfare, animal health and human/farmer health and safety concerns. The most common way of dealing with these challenges to date has been to disbud or dehorn cattle, but this does not eradicate the problem. Even after disbudding there is a chance of the approach failing and horns needing to be removed again in later life, and this coming with further production losses and economic cost. What-is-more, in commercial beef herds it may not be possible to disbud calves due to stock management constraints.

Many breeds of cattle, include both horned and polled varieties, hence the breeding of polled cattle may constitute a non-invasive option to replace disbudding. The identification and use of polled genetics in all NZ cattle would eliminate the costs, labour, animal health and welfare issues associated with the unpleasant procedure of disbudding or dehorning, while also making NZ dairy and beef production more efficient. In this context, the objective of this study is to validate a gene test, across five different beef and dairy breeds, identifying homozygous polled, heterozygous polled and homozygous horned animals.

2.1 The presence of horns

Cattle horn consists of dense keratin that is produced at the corium, the area of cells located at the junction of the horn and skin (Knierim *et al*., 2015). The horn buds start to form during the first two months of life, when they are free-floating in the skin layer above the skull. As the calf grows older, the horn buds attach to the skull, more precisely to the periosteum of the frontal bones overlying the frontal sinuses, and the bony horns then start to grow (Knierim *et al*., 2015). This begins around the age of 6 to 8 months. These are increasingly pneumatised from the caudal frontal sinuses, so that the hollow centres of the horn cores are directly connected with the frontal sinuses of the skull. The bony cores of the horns are supplied by blood vessels and nerves and will continue to grow during the entire life of cattle (Habel & Budras, 2003; Parsons & Jensen, 2006).

2.1.1 The potential phylogenetic functions of horns

In the past there have been many reasons given as to why cattle developed horns, with a large assortment of hypothesis being formed. One evolutionary benefit may be that they were of benefit in male intrasexual competition for mates (e.g. Preston *et al*., 2003; Bro-Jørgensen, 2007), but this does not justify why females would have evolved to have horns too. Estes (1991) suggested the hypothesis that male mimicry in female bovids, protects male offspring against the aggression from older and more dominant males, thus the sons will remain in the herd for longer. Additionally, Estes (1991) indicated that for male ungulates, horns serve as signals of genetic “quality” for female choice of mating partners.

Horns may have also provided advantages during evolution to use as defense against predators (e.g. Bro-Jørgensen, 2007; Stankowich and Caro, 2009) or in resource competition

(e.g. Roberts, 1996; Robinson & Kruuk, 2007). These functions are less relevant to cattle production settings in NZ, as males rarely need to compete for mates and predators are rarely if ever described.

2.1.2 The behavioural consequences of having horns

The presence of horns may affect the quality and quantity of social interactions between cattle, as well as social relationships in a herd, but literature explicitly dealing with social behaviour in horned herds in comparison to horn-less herds, is scarce and recent studies are lacking. The social behaviour research that has been undertaken in cattle does not state whether animals were horned or not.

The presence or absence of horns affects the way in which cattle can fight. During head-to-head pushing, horns have the function of hooking the animals together, thereby allowing a pushing force contest. In hornless cattle, due to the permanent slipping of foreheads, pushing force can only be exerted by neck/shoulder and the head is frequently used for hitting (Sambraus, 1978).

2.1.3 Management practises involved in horn removal

To date, a large proportion of cattle are disbudded or dehorned and in most cases without proper pain relief (Fulwider *et al*., 2008; Vasseur *et al.,* 2010; De Boyer des Roches *et al.,* 2014; Cozzi *et al*., 2015). It is a common practise on many dairy farms in NZ. This has been an area of interest due to increasing concern about animal welfare by animal rights groups and the wider public. Thus, alternative options might need to be considered to keep both the dairy and beef industries ahead of potential future regulations around horn removal or disbudding. There are also large costs involved with disbudding calves if pain relief is used, and this extra labour cost and product cost could be eliminated by producing hornless animals (refer to section 2.11).

2.2 Animal welfare in horned herds and disbudding

Disbudding of calves is a painful process that is carried out predominantly in the dairy industry. It involves the removal of the horn from the base of the skull. It is achieved by cautery (using a hot iron) or the application of a chemical paste. These approaches would be of interest to the public and animal rights groups specifically, on the grounds of whether

animal welfare regulations are being compromised or met. Disbudding is included in the Animal Welfare Act, 1999 (Stafford & Mellor, 2005) and the Painful Husbandry Procedures Code of Welfare, 2005 (Animal Welfare (Painful Husbandry Procedures) Code of Welfare 2005, n.d.). Dehorning is also common practise in both the beef and dairy industries. Involving the removal of horns later in life using horn cutters.

Alternative options to disbudding and dehorning need to be considered by the NZ cattle industries. These options might include using genetics to breed hornless cattle. Genetic approaches have been successfully applied to improve sheep welfare practises (Ferguson *et al.,* 2017), including the removal of horns in Merino rams (Van der Werf, 2012). The majority of this previous success has been based on gene marker and gene tests, but unless the underlying mutation can be eliminated from the population, such tests can become ineffective because of the constant recombination of the genome (Ferguson *et al.,* 2017). The main challenge for genetic solutions for the removal of horns in cattle is the limited accuracy of recorded phenotypes.

It is widely believed that the removal of horns from NZ cattle herds via genetics is not achievable, as there is a very small gene pool of polled dairy cattle, and thus there is a risk of losing genetic merit within herds. However, this may have been the case a decade ago, but bulls have since been bred that are polled and have moderate to high estimated breeding values (EBVs) and Breeding Worth (BW) (some are included in the LIC sires list) (The Spring bulls are here, n.d).

Disbudding induces stress and pain on to calves during and after the procedure, this can be assessed by an alleviation in behavioural responses, physiological responses and through measured live weight gain. Disbudding is commonly carried out without the use of post-operative analgesics. Post-operative pain is associated with behavioural (e.g. head rubbing, head shaking, ear flicking, vocalization) and physiological changes (e.g. plasma cortisol concentrations) that persist for at least 24 hours after the procedure (Stafford & Mellor, 2011; Faulkner & Weary, 2000; Stock *et al.,* 2013). A common indicator used to determine a calf's level of pain is plasma cortisol levels. Cortisol is a hormone released by the adrenal gland, that increases when stress is induced in an animal. Another indicator is the heart rate of an animal while undergoing a painful procedure. When an animal is stressed or in pain, their heart rate will increase, with this potentially lasting for prolonged periods.

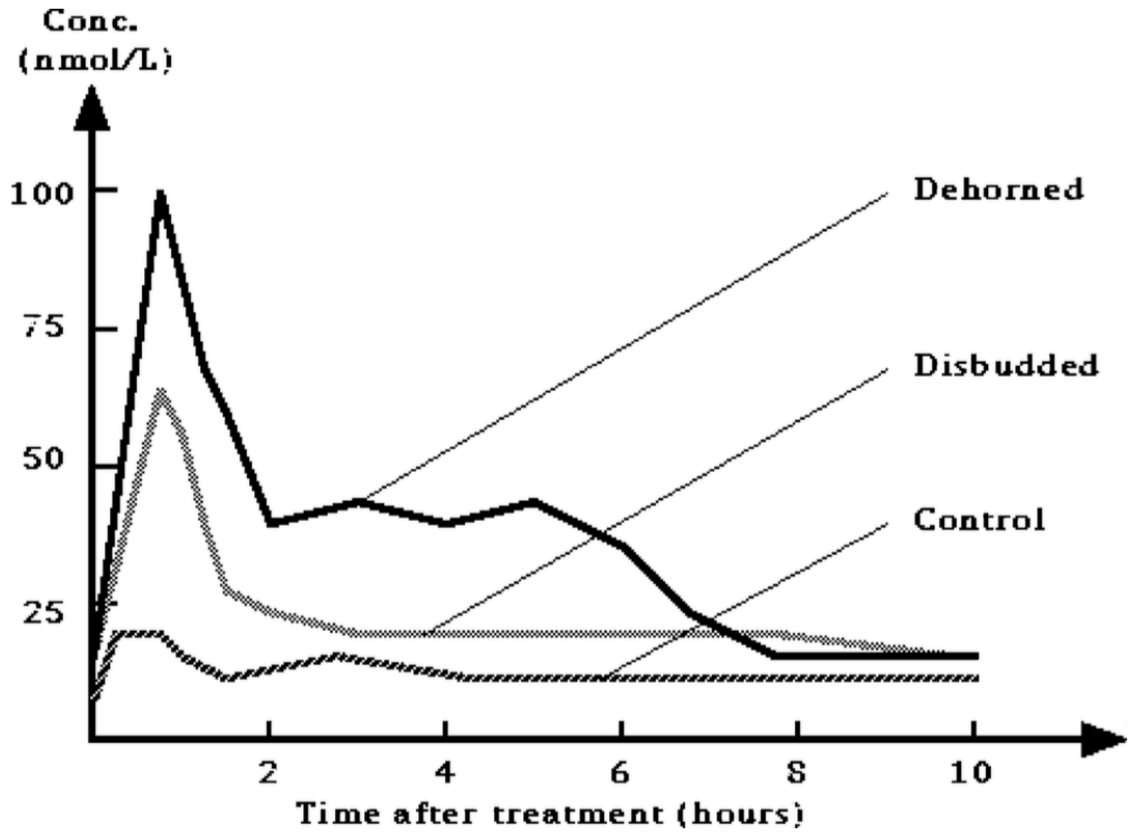

Figure 1: Changes in the plasma cortisol concentrations in 6–8 week old calves after amputation dehorning or cautery disbudding, and in control calves (sourced from Petrie *et al.*, 1996a).

During cautery disbudding, calves show distinct escape behaviour, including rearing, falling down, pushing, head jerking and moving, which are shown to be indicative of pain (Taschke & Folsch, 1993; Graf & Senn, 1999; Grondahl-Nielsen *et al*., 1999). There is a significant but short-lived cortisol response that peaks at about 30 minutes and is mainly complete within two hours (Figure 1, Petrie *et al*., 1996a). The cortisol response in calves shows that, for the first hour after cautery disbudding calves experience pain or distress greater than that experienced by the control calves, but after one hour this pain or distress has been alleviated or reduced greatly (Laden *et al*., 1985; Petrie *et al.,* 1996a). Salivary cortisol levels have also been found to peak 30 minutes after cautery disbudding (Taschke & Folsch, 1993).

The use of local anaesthetic prevents the obvious behavioral responses seen during cautery disbudding (Stafford & Mellor, 2011), but calves commonly resist its administration. Lignocaine, is a common local anaesthetic used, and while it prevents pain during the process of disbudding, the effect “wears off” and there is a subsequent resurgence of pain and distress (Stafford & Mellor, 2005), as shown by an increase in plasma cortisol

concentration. Induced pain can also be alleviated or prevented by a systemic analgesic such as the use of ketoprofen (Faulkner & Weary, 2000), or by cauterising the wounds after disbudding and preceded by lignocaine. Therefore, the use of pain relief can significantly reduce both behavioural and physiological pain responses from disbudding.

There has been speculation from farmers producing bull beef, as to what the potential production losses of disbudding with or without pain relief. It appears that there are no short-term or long-term effects of disbudding on food intake and growth rate of 4–6 and 8-week old calves (Laden *et al*., 1985; Grondahl-Nielsen *et al*., 1999). However, this is contradictory to research conducted at Massey University in 2015-2016, revealing that an increase in the use of pain relief prior to disbudding will not only reduce pain and recovery time but also avoids a growth check. Massey University (Dairy NZ, 2017) revealed that calves that receive pain relief prior to disbudding went on to achieve higher growth rates. In this trial the calves on average gained 0.09kg more per day for the next month, thus reaching weaning weight five days earlier than calves that didn't receive pain relief (Dairy NZ, 2017). With earlier weaning, less milk needs to be fed, less labour is required, and calves are moved onto grass and meal sooner. The reduced costs and the benefits of having heavier calves easily offsets the cost of pain relief.

2.3 Genes invovled in polled and horned cattle

Bateson and Saunders (1902) were the first to report that the polled condition in cattle was dominant over the horned condition. This is supported by Williams and Williams (1952) revealing results from crossing polled Herefords and horned Herefords. Furthermore, Lloyd-jones and Evvard (1916) also demonstrated the dominance of the polled gene, in Shorthorn bulls mated to Galloway cows. This was further supported by various studies (Barrington & Pearson, 1906; Spillman, 1906) that confirmed a single-gene means of horn inheritance.

2.3.1 Location of *Polled* locus

The *Polled* locus has successfully been mapped to the centromeric region of BTA 1 (Georges *et al*., 1993; Schmutz *et al*., 1995; Brenneman *et al*., 1996; Harlizius *et al*., 1997; Drogemuller *et al.,* 2005; Mariasegaram *et al.,* 2012; Seichter *et al*., 2012). In various beef and dual purpose cattle breeds an apparent causative mutation for polledness occurs. In cattle of Celtic origin it has been described as a structural sequence variant, a complex insertion-

deletion affecting an intergenic region of BTA 1 (explained in figure 2) (Medugorac *et al*., 2012; Wiedemar *et al*., 2014). High-density SNP genotyping confirmed the presence of two different poll associated haplotypes in Simmental and Holstein-Friesian cattle that co-localized on BTA 1. These are the Simmental *Polled* locus being refined to 212kb in the centromeric region of BTA 1 and an overlapping region containing the Holstein *Polled* mutation, refined to a 932 kb region (figure 2) (Weidemar *et al*., 2014). Medugorac *et al*., (2012) identified a single complex insertion-deletion event (P_{202}ID) that was perfectly associated with the polled gene in most European cattle breeds, confirming the recently published possible *Celtic Polled* mutation. After fine mapping of the entire chromosome segment, there was an absence of any other congruent candidate variants. This was further supported by a perfect association with phenotypes, high sequencing conservation of the candidate fragment among horned mammals and the absence of the appropriate candidate fragment in polled mammals (Medugorac *et al*., 2012). This clearly suggesting P_{202}ID is most probably the casual mutation for polledness in most *Bos taurus* breeds.

Genetic markers have been used to implement marker-assisted introgression and increase the polled gene in breeding populations, even before the precise location of the polled gene was known. Georges *et al*., (1993) demonstrated a genetic linkage between the *Polled* locus and two microsatellite markers (GMPOLL-1 and GMPOLL-2) in *Bos taurus* cattle, and assigned these markers to bovine chromosome 1 (BTA1). These markers were subsequently reported to be TGLA49 and AGLA17, respectively (Brenneman *et al*., 1996). At a molecular level these early studies confirmed the existence of a "*Polled*" locus and a clear inheritance pattern. This laid the foundation to search for closer markers to "*Polled*" in an attempt to more effectively trace the segregation of the true polled gene.

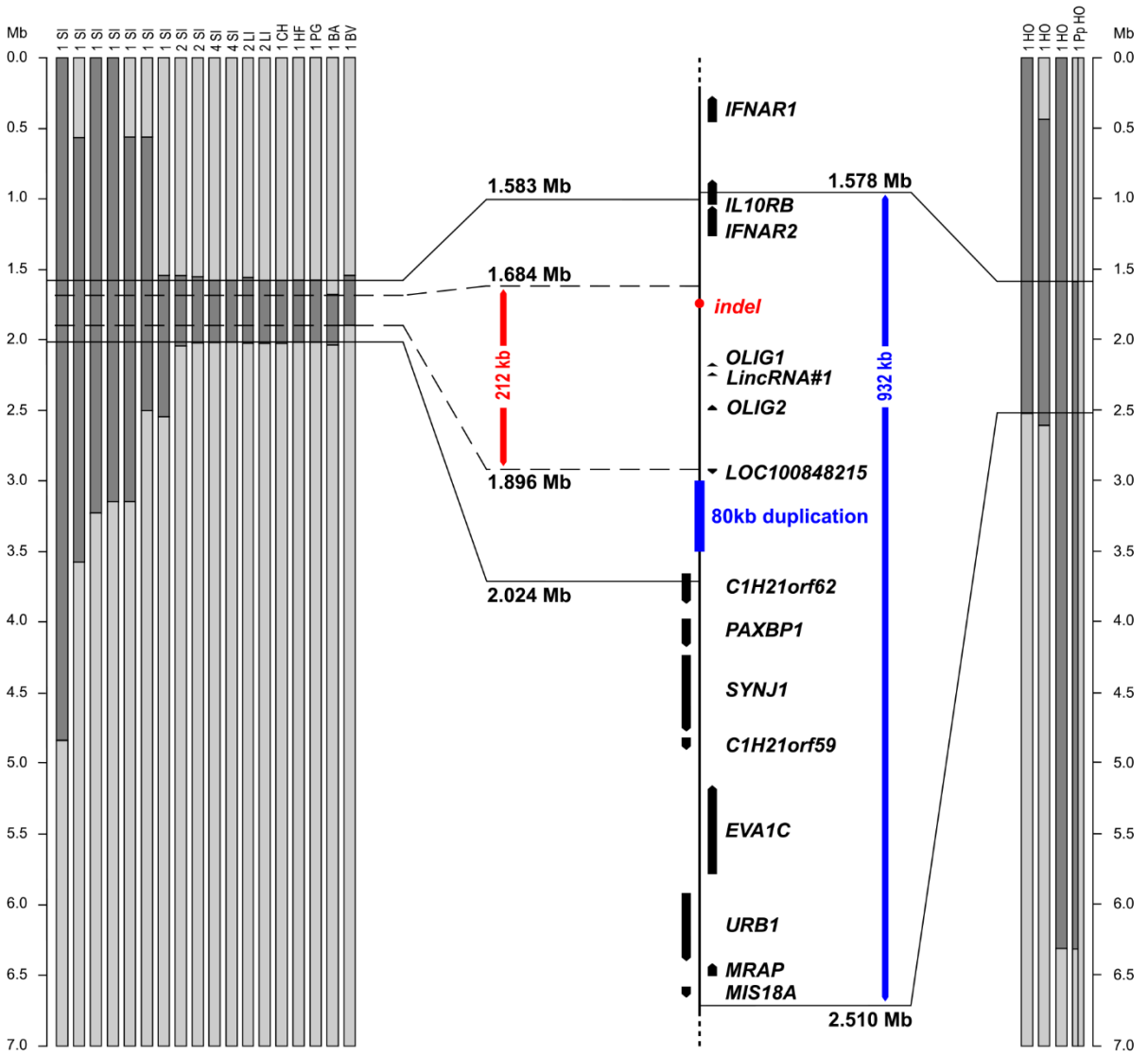

Figure 2: Homozygosity mapping on BTA 1 (sourced from Wiedemar *et al.,* 2014).

The vertical bars represent the SNP genotypes of BTA 1 markers, the dark grey segments represent homozygous blocks with shared alleles. The annotated genes and loci on the BTA 1 segment (UMD3.1 assembly) are shown in the centre. Above the chromosome bars are the number of animals, belonging to beef and dual-purpose breeds of Celtic origin (SI: Simmental, LI: Limousin, CH: Charolais; HF: Hereford, PG: Pinzgauer; BA: Blonde d'Aquitaine, BV: Braunvieh). Shown in red is the suggested position of the Celtic *Polled* mutation within a 212 kb interval. The blue area indicates the critical region of the Friesian *Polled* mutation (932 kb) (Wiedemar *et al*., 2014).

The availability of linkage maps (Barendse *et al*., 1994; Bishop *et al*., 1994) led several researchers to investigate markers linked to the polled gene. Schmutz *et al*., (1995) mapped the *Polled* locus close to the centromere of bovine chromosome 1 in five Charolais families.

LOD scores (a statistical estimate of whether two genes, are likely to be located near each other on a chromosome, and therefore likely to be inherited) indicated 100% linkage between microsatellite markers (TGLA48 and BM6438) and the polled phenotype. There was then a large amount of research (Brenneman *et al*., 1996; Georges *et al*., 1993; Harlizius *et al*., 1997) conducted to localise the position of the *Polled* locus, but these efforts were unsuccessful.

Recent advances in molecular genetics, has resulted in the refined location of the *Polled* locus. However, there is large scope for new research to be conducted, developing an understanding of possible relationships and confounding effects between the polled, scur and African horn genes. Leading to the development of genetic tests able to identify homozygous/heterozygous animals for polled, scur and African horn genes (Prayaga, 2007). Therefore, assisting to increase the introgression of the polled condition in NZ cattle herds.

2.3.2 Scur locus

A second locus affecting horn growth in cattle is called scurs (White & Ibsen, 1936; Long & Gregory, 1978). Scurs are corneous growths of different sizes from crusts up to big horn-like formations, which develop in the same area as horns, but that are not firmly attached to the skull. Dove (1935) studied the physiology of horn growth and concluded that the horn core is due to a separate centre of ossification originating in the tissues above the periosteum, fusing to the skull and thereafter appearing as a simple exostosis. This study also clarified that scurs have a bony core at the distal end and at the same time have a boney deposit on the skull at the base of the scur. This deposit at the base of the scur can extend a short distance, known as loose scurs. It can extend far enough to form rigidity (ridged scurs), but will not reach the bony core present at the distal end.

In Angus and Galloway cattle, scurs have been described to develop depending on the sex and polled genotype (Long & Gregory, 1978). Homozygous polled animals develop scurs only if they also carry the Sc mutation (scurs mutation) in a homozygous state, heterozygous polled females develop scurs only if they carry the Sc mutation in a homozygous state. In comparison heterozygous polled males develop scurs in the presence of one or two Sc alleles (Long & Gregory, 1978).

However, these associations are not supported by Wiedemar *et al*., (2014) who found that 207 scurred animals were heterozygous for one of the polled mutations, indicating epistasis of the *Polled* and *scur* loci. Thus, *Polled* is epistatic over *scurs* and in homozygous polled animals scurs cannot be expressed, as 191 homozygous polled cattle showed no signs of scurs (Wiedemar *et al*., 2014). However, the mode of inheritance of the scurs mutation is still under debate (Long & Gregory, 1978; Capitan *et al*., 2009), due to factors such as age having an effect on the time in which scurs are present, shown from a skull dissection made by Brenneman *et al*., (1996). This mode of inheritance and the expression of phenotype being influenced by age of the animal complicates any form of study of the inheritance based on phenotypes. Thus, a definitive gene test for differentiating scurred, horned and polled animals is required to make accurate breeding decisions.

2.4 **Inheritance of polledness**

Williams and Williams (1952) described the horn phenotypes in Hereford cattle, supporting White and Ibsen's, (1936) theory of four pairs of alleles controlling the polled/horned/scurred phenotypes. Horns vary in length and shape, from short curved horns to large sweeping horns. Tight scurs are considered to be short stubs, which are firmly attached to the frontal bone. Loose scurs are the same as tight scurs except being smaller and attached to the skin rather than the frontal bone. The round polled (poll being the central prominence on the head) phenotype is where the skull between the horns is rounded, with a slight protruding horn loci in most individuals. The peaked polled phenotype refers to animals that display the centre of the frontal eminence as peaked and not rounded. The Williams and Williams (1952) study revealed that peak polled animals much more reliably produced completely polled animals than others.

Long and Gregory (1978) investigated the inheritance of the horned, scurred and polled conditions in a study involving 830 progeny from various Angus (polled), Polled Hereford, and Horned Hereford sires. They concluded that the single-locus model with multiple alleles did not explain inheritance adequately and that the inheritance model proposed by White and Ibsen (1963) of four separate loci was generally more consistent with their results.

The horned and polled trait in cattle is considered to be determined by one pair of genes, however these genes have been shown to be breed specific (Mariasegaram *et al.,* 2012; Wiedemar *et al*., 2014). One gene in the pair is inherited from the dam and the other from

the sire (Allison, 1996). The polled gene (P) is dominant to the horned gene (p). If an animal has two polled genes (PP), homozygous, or one polled and one horned gene (Pp), heterozygous, it will be polled. However, if it is heterozygous polled (Pp) it may pass either the polled or horned gene on to its offspring (table 1). The only situation when an animal will be horned is when it possesses two recessive horned genes (pp), homozygous horned (Allison, 1996). However, there are additional genes that affect horn-like growth and scurs that appear on animals' heads. This includes effects on the shape, size and orientation of horns being under the influence of many genes, each with minor effects like any other quantitative trait (Warwick & Legates, 1979).

Table 1: Genetic Expression of Polledness or Horns and Expected Inheritance by Offspring

Sire	Dam	Calves
Homozygous polled (PP)	Homozygous polled (PP)	100% Homozygous polled (PP)
Homozygous polled (PP)	Heterozygous polled (Pp)	50% Homozygous polled (PP) 50% Heterozygous polled (Pp)
Homozygous polled (PP)	Homozygous horned (pp)	100% Heterozygous polled (Pp)
Heterozygous polled (Pp)	Homozygous horned (pp)	50% Heterozygous polled (Pp) 50% Homozygous horned (pp)
Heterozygous polled (Pp)	Heterozygous polled (Pp)	25% Homozygous polled (PP) 50% Heterozygous polled (Pp) 25% Homozygous horned (pp)

If an animal from European breeding has horns, it can be determined as homozygous horned by visual assessment. However, an animal with a smooth polled head or that is scurred cannot be visually determined to be homozygous polled or heterozygous polled. Thus, the best way to determine the polled genotype is through using a genetic test to identify heterozygous animals, especially when trying to eradicate horns from a herd.

Another factor that complicates the inheritance of polledness is that in cattle with Zebu ancestry such as Brahman and Santa Gertrudis, there is an additional gene associated with the inheritance of horns (Allison, 1996). Inheritance of horns in Zebu-type cattle is different

from what is observed in British breeds. The polled gene (P), and the scur gene (Sc) can both be present in American cattle with Zebu ancestry. However, another gene, the African horn gene (Af) also affects inheritance of horns in these animals. Where the African horn gene is expressed is dependent on the gender of the animal, as is the case for scurs (Allison,1996).

Due to the mode of inheritance through three main loci, polled, scurs and African horn, and the sex-influenced nature of inheritance coupled with epistatic effects (Prayaga, 2007), the horned phenotype is not a suitable determinant for making breeding decisions to propagate polledness in cattle. Thus, it is important to know the homozygous and heterozygous state at these loci to effectively reduce the proportion of horn alleles in a breeding population while monitoring the masked scur phenotype, as the scur gene does not express in horned animals, even in the dominant homozygous state (Prayaga, 2007). Propagation of the polled gene in purebred herds is inhibited due to the inability to distinguish what animals are heterozygous or homozygous. This is where genetic testing would be advantageous.

2.5 Relationship between Polledness and productive attributes

2.5.1 Economically important traits

Many traits such as growth, fertility and longevity are significantly important for beef production. No significant differences have been reported in liveweight in Shorthorns (Marlowe *et al*., 1962) and in mortality rates in Herefords (Longland *et al*., 1976), in the context of them being horned or polled. Although an earlier study by Wythes *et al*., (1976) reported a higher incidence of dystocia in polled Herefords than in horned Herefords, this is based from survey data without any adjustments for management practises. Frisch *et al*., (1980) found no significant differences between horned and polled cattle in liveweight, fertility, or mortality rates, indicating that polledness had no detrimental effect on production in tropically adapted genotypes such as tropically adapted Hereford-Shorthorn cross (100% *Bos taurus*), Brahman cross (50% *Bos taurus* and 50% *Bos indicus),* and Africander cross (50% *Bos taurus* and 50% tropically adapted *Bos taurus)*.

In Canada, Stookey and Goonewardene (1996) reported no disadvantage for polled bulls compared with horned bulls, on-test average daily gain, weight per day of age, adjusted scrotal circumference, and adjusted yearling weight in Charolais and Hereford cattle. Goonewardene *et al*., (1999) reported no differences between horned and polled cattle in three beef synthetic lines for various growth and reproductive traits. These include

pregnancy, calving and weaning rates, calf birth and weaning weights (Pang *et al.,* 1998), calf pre-weaning average daily gains, dystocia score, cow weights and cow condition scores at calf birth and calf weaning. The similarities in growth and reproduction traits agrees with those of Frisch *et al*., (1980). Goonewardene *et al*., (1999) also reported similar growth and majority of carcass traits between polled and horned composite bulls. The differences between polled and horned cattle observed and reported by producers are often confounded by the intensities of selection placed on different traits, which vary between farms, due to differences in management practices.

Although many studies suggest the phenotypic differences in traits associated with reproduction, growth and the carcass, between horned and polled cattle are small and of little significance (Frisch *et al*., 1980; Lange *et al*., 1990; Stookey & Goonewardene, 1996), there has been a tendency on the part of pure breeders to keep horned and polled populations separate (Koots & Crow, 1989).

2.6 **Relationship between Polledness and bull reproductive attributes**

Increasing the prevalence of the polled gene through the beef industry has come with significant benefits, but concerns around breeding and fertility of polled bulls have been of interest. Perceptions have been summarised by Milne (1954) regarding bull fertility. These include (1) the perception that the polled gene is associated with an increased prevalence of the condition of premature spiral deviation of the penis (PSDP), (2) the perception that the polled gene is associated with an increased prevalence of preputial prolapse, (3) the perception that polled bulls have reduced serving capacity (libido) compared to horned counterparts.

2.6.1 Premature spiral deviation of the penis

In the past, premature spiral deviation of the penis has been associated with the polled gene, especially in Hereford cattle. Spiral deviation occurs when the erect free end of the penis of an affected bull spirals to the right hand side in an anticlockwise direction (Blockey & Taylor, 1984). The effective width of the penis is doubled thus preventing intromission (Ashdown & Pearson, 1973). Therefore, affecting the serving capacity of the bulls affected. This defect is more prevalent in beef rather than dairy breeds (Pearson & Ashdown, 1974) and polled bulls were more commonly affected than horned bulls (Milne, 1954; Whitsell, 1969; Pearson & Ashdown, 1974). However, this research was conducted 25 to 50 years ago

and an insufficient amount of data was found to support that PSDP is more likely to occur in polled cattle. Norman *et al*., (2009) determined that there is a lack of evidence suggesting that PSDP is directly associated with the polled gene, but is simply recognised more frequently within the polled breeds. This may be more a reflection of the initial gene pool of these breeds, rather than a direct link to the polled gene. Where selective breeding occurred to target the polled phenotype but lost other productive traits. This idea is supported by an anatomical study conducted by Ashdown and Pearson (1973), providing circumstantial evidence that the condition could be heritable (three of the eleven bulls affected, were related polled Hereford bulls). This leading weight to considering the PSDP to be a breed condition rather than specifically related to the polled gene. However, the heritability of PSDP is still not well understood, with little statistical data to confirm heritability.

A study by Blockey and Taylor (1984) implicated a link between the polled condition and PSDP. This study was based on the serving capacity data from over 1,000 bulls, including 415 Angus bulls, 167 polled Hereford bulls and 448 horned Hereford bulls. With the remainder consisting of polled Shorthorn, Red Poll and Murray Grey bulls. The prevalence of PSDP in the polled breeds compared to horned breeds was 16% and 1%, respectively. In polled Hereford and horned Hereford bulls the prevalence was 10% and 1%, respectively. When Blockey and Taylor (1984) examined the relationship of affected Angus bulls, they found a high degree of common ancestry between bulls at the first, second and third generation. This supports the concept of possible heritability of PSDP. Nonetheless, it also supports that PSDP could be breed specific. However, apart from Blockey and Taylor (1984), there is little scientific evidence to support a specific association between the polled gene and PSDP. Based on the current data it is theoretically possible that the polled gene or a DNA fragment influenced by the polled gene, could modify the structure or function of the dorsal apical ligament of the penis (Norman *et al*., 2009).

2.6.2 Preputial prolapse

The polled gene has been strongly linked to a deficiency in the development of the caudal preputial muscles (responsible for protracting the prepuce (Penis - Anatomy & Physiology, 2012)), and a higher prevalence of preputial eversion in polled *Bos taurus* breeds (Bruner & Camp, 1992; Long & Hignestt, 1970; Rice, 1987). Chronic prolapse of the parietal layer of the prepuce (skin sheath that conceals the penis (Penis- Anatomy & Physiology, 2012)) of bulls may lead to preputial injuries and related breeding difficulties (Lagos & Fitzhugh, 1970).

Study numbers are usually small, however a study by Long and Dubra (1972) was of significant scale, comparing 275 polled bulls to 210 horned bulls, the reported prevalence of preputial eversion in polled versus horned animals was 81% and 64%. From this almost 70% of the 169 Hereford bulls everted their prepuce, suggesting that there is a relationship with the polled gene. However, its influence on the development of the caudal preputial muscles is not the only factor determining the occurrence of preputial eversion. The only anatomical differences between the prepuces of polled and horned bulls is that horned bulls have a significantly larger mean volume of prepuce compared to polled bulls (Long & Hignett, 1970).

The link between preputial eversion and preputial prolapse is relatively unclear, with some work strongly suggesting that the frequency of eversion is not related to the prevalence of clinical prolapse in *Bos taurus* breeds (Long & Dubra, 1972). A number of factors have been involved in the aetiology of preputial prolapse (Venter, 1978), including injury, external parasites, infection, functional and anatomical abnormalities of the prepuce and caudal preputial muscle, inefficient caudal preputial muscle action, pendulous sheath (Wolfe *et al*., 1983), size of the preputial orifice, longer preputial length, excessively pendulous sheath and angle of the opening of the sheath (Ashdown, 2006) and genetic predispositions. It has been suggested that *Bos indicus* and polled breeds in general have increased susceptibility to the condition (Venter, 1978). While there appears to be strong data suggesting polled bulls have abnormal caudal preputial muscle development, it is also apparent that management conditions and sheath structure play an important role in the development of the pathological preputial prolapse.

In the study conducted by Venter (1978) there is evidence of an increased prevalence of preputial prolapse associated with the polled gene and reduced development of the caudal preputial muscles in a *Bos indicus* derived species. This is in contrast to the apparent situation in *Bos taurus* species. Although it has been suggested that *Bos taurus* species are considered less susceptible to preputial prolapse compared to *Bos indicus*, the Hereford breed has been identified as the most susceptible to prolapse in the *Bos taurus* species (Roberts, 1956). However, preputial prolapse is extremely rare in Shorthorns and their crosses (Lagos & Fitzhugh, 1970), with only a low prevalence being recorded in cross-bred Santa Gertrudis bulls where the maternal grandmothers were Shorthorns (Venter, 1978). Thus, there is an area of research needed to identify any relationships between bull

soundness and the polled gene in beef cattle. Based on the current research the presence of the polled gene is unlikely to increase the prevalence of preputial prolapse, due to a large range of contributing factors.

There is no evidence supporting the association between the polled condition and reduced serving capacity (Norman *et al*., 2009). Frisch *et al*., (1980) found no relationship between cryptorchidism (one or both testes undescended) in cattle. Frisch *et al*., (1980) also found no evidence to support that horned males were more fertile than polled males, contradictory to the perceived association between horns and maleness.

2.7 **Previous Gene tests identifying polled and horned status**

Currently there are a number of gene marker tests available identifying the horn status in cattle, however these are not in NZ. Gene marker tests are similar to estimated breeding values (EBVs), where EBVs estimate all the genetic variation and the specific sources of variation (genes) are unknown. Gene marker tests reveal the genotype of an animal for specific gene markers for a particular trait but do not account for all of the genetic variation. The use of gene marker information can allow for early prediction of the genetic merit of an animal before phenotypic records are collected, thus increasing the accuracy of young sires and decreasing generation interval.

Gene marker tests for horned status allow for a producer to determine if a polled animal is homozygous polled or heterozygous polled (carrier of the horned allele). All horned animals are homozygous for the horned allele while animals that have a polled phenotype may be carriers of a horned allele and produce horned offspring if mated to females who are horned or heterozygous polled. Different companies have validated tests covering different breeds. Breeds that have tests available include Charolais, Gelbvieh, Hereford, Limousin, Salers, Simmental, and Tarentaise. The tests currently available are predominantly available through the Animal Genetics laboratory at the University of Queensland and Zoetis Animal Genetics.

The Australian Poll Gene Marker test was first developed by the Beef Cooperative Research Centre (CRC) and released commercially in 2010. The initial test was based on a single DNA marker and worked accurately within some breeds, with accuracy issues in other breeds. In 2013, a newly improved indirect Poll Gene Marker test was developed and released, using

ten gene markers associated with polledness. This test has nearly 100% accuracy across Brahman, Santa Gertrudis, Tropical composite, Brangus, Droughtmaster, Hereford, Limousin, Shorthorn, Simmental and Charolais (Meat and Livestock Australia, 2013). The research conducted to date will return an informative result for the vast majority of animals tested (table 2).

Table 2: Number of polled animals tested and proportion of genotypes assigned with confidence (% non-ambiguous) for nine breeds assessed during polled marker field and commercial testing. (Sourced from Meat and Livestock Australia, 2013)

Breed	Number Tested	Informative results
Brahman	299	84%
Brangus	104	89%
Charolais	65	89%
Droughtmaster	102	77%
Hereford	174	96%
Limousin	297	95%
Santa Gertrudis	225	92%
Shorthorn	167	94%
Simmental	118	93%

Former Meat and Livestock Australia CEO Scott Hansen stated that "Using normal breeding practices it would take 39 years (to remove horns), but the new Poll Gene Marker test reduces that down to eight years" (ABC News, 2014). The test has a cost of AU$25, however to test a bulls DNA and only pay AU$25 should be considered a good investment. It is a one off investment that flows through to future generations.

Another DNA test available for determining the horned/polled genotype in beef cattle is 'HornPoll'. This is another Australian product from Pfizer, that is used to identify the probability of an animal carrying zero, one or two copies of the polled variant gene (Pfizer, 2014). The HornPoll test is breed-specific based on the utility of the test for particular breeds validated by the Beef CRC (2011). The HornPoll test is only suitable for Brahman, Santa Gertrudis, Droughtmaster, Hereford and Simmental breeds, and any crosses of these breeds (Pfizer, 2014). Based on the HornPoll test, the report includes a probability of an animals reported genotype, reflecting the horned/polled status of that animal. The probability estimates are given as a range between 0 - 100%, based on Beef CRC validation results, a

higher value represents an increased level of confidence in the result (Pfizer, 2014). A new version of this test has been developed and was advertised by Zoetis Australia, in 2014. The new version was developed to reduce the incidence of unresolved genotypes occurring with the previous version, providing increased confidence across a range of temperate and tropical breeds produced in Australia (Zoetus, 2014). The HornPoll test can be performed using hair, semen or other tissue types regularly used for DNA analysis.

It is clear that there is ongoing research of the genes and markers involved with the polled variant, thus further development of DNA or gene tests will occur resulting in the test being regularly revisited. This will keep occurring over time as technology keeps evolving and improving. However, it is beneficial to have current tests on the market to keep the beef and dairy industries advancing.

2.8 Breed Backgrounds

All breeds investigated were of British origin. However, all breeds have diverged separate ways and carry varying characteristics. Each breed has been bred for specific areas of production, for example beef or milk production.

2.8.1 Hereford

Hereford cows contribute to 14% of the one million breeding cows and heifers in the NZ beef herd (Beef Industry Overview, 2017) and are a favourable choice for cross-breeding in commercial farming systems. Gregory *et al.,* (1966) revealed that Hereford cattle contribute more than either Angus or Shorthorn to the average heterosis effects when crossed, with Herefords ranking highest in net merit (calculated by carcase composition and fed efficiency). In commercial NZ beef herds cross breeding is common, as these cattle tend to grow out more efficiently (Gregory *et al.,* 1966) and provide quality carcasses (Gregory *et al.,* 1966). Hereford cattle have long been used for both milk and beef production, currently Hereford cattle are primarily bred for beef production.

The Hereford breed originated from Herefordshire in the United Kingdom (UK), in the middle to late 1700's. Their ancestors consisted of cattle which were native to Britain in that time period, but may have also included cattle from other regions of Europe (Heath-Agnew, 1983). The Hereford breed has been under selection for more than 150 years. Hereford

cattle are a pre-eminent breed that has proved successful in adapting to many environments and is now found in several countries throughout the world (Blott, Williams & Haley, 1998).

The first importations into NZ of pedigree Herefords (horned) arrived from England by sailing ship in 1869, brought in by R & E McLean. Not long after the NZ Hereford association was formed in 1896 by J Stuckey, G C Wheeler and D P Buchanann (NZ Hereford Association, 2017). Today, over 400 breeders are registered, taking advantage of the pure blood lines. Early Herefords were naturally horned until 1929 when the hornless variants with the polled gene were introduced to NZ, known as polled Herefords. Hereford breeders began performance recording with Breedplan at the Agricultural Business Research Institute (ABRI), in Armidale, Australia in 1992 (NZ Hereford Association, 2017). The NZ Hereford breed advanced in 1993 with the launch of Hereford Prime NZ Limited branded beef.

Herefords are a medium to large sized muscular breed, with a prominent appearance, red body and white face. They have good maternal qualities, both as terminal sires and for cross-breeding. The Hereford breed is known to be highly fertile and for having a docile temperament, allowing easier handling than other cattle breeds (NZ Hereford Association, 2017). Meat quality is high, rivalling that of Angus, another British breed, known for its 'marbling' attribute (Reverter *et al.,*2000). The ability of the breed to adapt to vastly differing environments is a true testament to the hardiness of the breed that, while originating in cool moist Britain, they have found great success, and indeed have thrived, in much harsher climates (NZ Hereford Association, 2017).

2.8.2 South Devon

The South Devon breed originated in South West England (British origin), in an area of Devon known as South Hams, from here they spread across the counties. Historical evidence indicates that isolation caused the divergence of North and South Devon into physically distinct types (South Devon, n.d.).

They are known for their rapid growth, without impairing the quality or quantity of the milk produced. The South Devon as a meat producing breed begun to achieve world-wide prominence during the 1960's when weight-gain recording became popular (About the Breed, n.d.). The South Devon breed was first introduced to NZ in 1969 (About the Breed,

n.d.), with their role in cross-breeding for beef herd improvement being firmly established, with stud's nation-wide supplying bulls for this purpose. Their quiet temperament makes them easy to handle and work with. By the 20th century South Devon were considered as a triple purpose animal, for production of beef, milk and butterfat.

South Devon's are a distinct breed having strong curly copper-red coats and pink around the eyes, nose and muzzle (About the breed, n.d.). The breed was predominantly horned although many naturally polled animals exist (South Devon, n.d.). Polled numbers are increasing due to active selection. South Devons are the largest of the British breeds, having a large frame and muscular conformation. They have broad heads leading into a deep body, where a mature bull can weigh approximately 1200kg to 1500kg (South Devon, n.d.). Bulls can be used as pedigree, crossing and terminal sires. South Devon females are early maturing and can be calved at 2 years of age. South Devons have been shown to yield leaner carcasses than other British Breeds and have been found to produce high levels of marbling (The Grove Herd, n.d.).

2.8.3 Belgian Blue

Belgian Blue cattle are a beef breed that originated in central and upper Belgium. Belgian Blue are considered a large breed of cattle with rounded outlines and prominent muscles. Their colour can range from white, blue roan, black or a combination of these. Belgian Blue cattle are well known for their shoulder, back, loin and rump being heavily muscled (Breeds - Belgian Blue, n.d.).

Belgian Blue cattle have a visibly distinct hypertrophy (mh), commonly known as double-muscling, as result they are often selected for their superior muscling ability. The autosomal recessive *mh* locus causing double-muscling condition in these cattle maps to bovine chromosome 2 within the same interval as *myostatin,* a member of the TGF-β superfamily of genes (Kambadur *et al*., 1997). Therefore, this gene suppresses the production of myostatin, a protein that normally inhibits muscle growth after a certain point (Kambadur *et al*., 1997). Pure Belgian Blue carry two copies of the gene. When crossed with other breeds, one copy is usually inherited and serves the purpose of increasing the carcass weight of offspring.

The active selection for this dual purpose type of animal started in 1920 and 1950 (in Belgium) (Breeds-Belgian Blue, n.d.). There was a breakthrough in the 1960's with the development of the extreme double-muscling characteristics. Belgian Blue cattle are not commonly used as a maternal animal, due to calving issues that arise with double muscling (Bellinge *et al*., 2005). They are used as a terminal cross, to target the carcass composition and the growth potential Belgian Blue cattle are renowned for.

2.8.4 Shorthorn

Shorthorns have made an important contribution to dairy and beef industries worldwide. The Shorthorn breed has evolved over the last two centuries, from Teeswater and Durham cattle found originally in the North East of England in the Tees river valley and Durham (Shorthorns, n.d.). The first Shorthorn cattle to arrive in NZ, still known at that time as Durhams, were introduced by Samuel Marsden in 1814 (Milk Shorthorn Cattle, n.d.). Shorthorns were used in the early part of the 20th century primarily as a dual purpose breed, but specialisation for beef and milk production led to the beef breeders starting their own section of the herd book in 1958. This led to the diversion of the beef and dairy shorthorn.

The dairy breeders sought to improve the dairy aspect of Shorthorns, thus a blending scheme to introduce outside blood from other breeds was introduced in 1970 (Milk Shorthorn Cattle, n.d.). Some breeders selected not to participate, causing large diversity within the breed. The Shorthorn breed has been important in the development of other breeds, with Shorthorn genetics being used world-wide in the development of over 40 different breeds.

Dairy Shorthorns are either red, red and white or white or roan. They have moderate frames, weighing between 635-990kg (Shorthorn, n.d.). The term 'shorthorn' refers to the cattle as having short horns, opposed to the medium or long horns seen in other cattle breeds. However, there are polled Shorthorn cattle, due to selection pressures favouring polled animals.

Dairy Shorthorns have small calves compared to other breeds. The calves are vigorous at birth and are considered to be hardy. Dairy Shorthorn cows recover quickly from calving and are in condition to re-breed earlier than most other dairy breeds (Shorthorn, n.d.). Dairy

Shorthorns are known for their milk having a favourable protein-fat ratio, ideal for making cheese. Shorthorns are also known for their structural soundness and longevity. Most cows are productive for five or more lactations, and dairy Shorthorn cows have been found to produce in excess of 10,000 kgs milk per lactation at greater than ten years of age (Shorthorn, n.d.).

2.8.5 Holstein-Friesian

NZ Holstein-Friesians originated from the first importation of the 'Dutch-Friesian', made by Canterbury breeder JCH Grigg in 1884 (Holstein-Friesian cow, 2017). Four years later the breed was introduced to the North Island via a herd established in the Wairarapa. Further importations of 'Holstein-Friesian' cattle were made from America in 1902/03 (Holstein-Friesian cow, 2017). The national dairy herd now contains about 40% North American Holstein (Holstein-Friesian cow, 2017).

The large framed, black and white dairy cows quickly gained popularity in the North Island, but it wasn't until 1910 that their breeding and importance was taken up intensively. Early endeavours by breeders to keep accurate pedigrees of these animals resulted in the NZ Holstein-Friesian Association being established in 1910 (Holstein-Friesian cow, 2017). In the 2015/16 season the average litres of milk per cow was 6,194 from a pasture-based diet (Holstein-Friesian cow, 2017).

Holstein-Friesians are predominantly horned animals. It is common practise in NZ when calves are young (6-8 weeks of age) to have their horns removed, this is known as disbudding. Allan McPherson, LIC (Livestock Improvement Corporation) Breeding Manager stated that "Historically NZ farmers interested in breeding polled Holstein-Friesian cattle had to compromise with a lower BW (Breeding Worth)" (First Polled AI Sire, n.d). This has been a common perception and has slowed the progress of integrating polled sires into the dairy market. LIC has been pursuing the polled gene for a decade and has had some partial success in the past. In 2012 the first polled Holstein-Friesian (Poll Axe) successfully graduated to be an Artificial Insemination (AI) sire. It was bred by the Coster family of Kaimai (First Polled AI Sire, n.d). Polled Holstein-Friesians are still the minority in the NZ dairy industry.

2.9 **Breed Variations**

Within the beef industry polled genetics have been widely adopted, with breeds such as the Angus and Galloway breeds are fully polled. This is expected to be due to divergence and selection for polled animals being more intensive in these two breeds, when compared to other beef breeds.

In the dairy industry the polled gene has not been widely adopted, because it is perceived to be associated with production losses. The dairy industry widely uses disbudding and de-horning as the primary management tools for reducing horn development in dairy herds.

Breeds such as the Angus are fully polled. The NZ Angus originated from the polled Aberdeen-Angus, but how the polledness originated and descended will probably never be satisfactorily determined, as there are no records of any ancient date, the best authorities offer only suggestions. It does seem most likely that three beef breeds of Scottish cattle, the polled Aberdeen-Angus, the Galloway and the West Highlander, descended from one stock: that of the wild aboriginal cattle of ancient Caledonia (History of Angus, n.d).

There are areas where breed differences occur in terms of the position of the *Polled* locus. This variation has been shown between Simmental, other beef breeds and Holstein-Friesian cattle. The *Polled* locus is positioned on chromosome 1 (BTA 1). The breed variation is in the position in which the gene is present on BTA 1. High-density SNP genotyping has been used to identify different polled associated haplotypes co-localized on BTA 1. In Simmental cattle and other beef breeds the *Polled* locus has been refined to being in a 212 kb fragment of the chromosome and it overlaps with a 932 kb fragment containing the Holstein Friesian *Polled* mutation (Wiedemar *et al*., 2014). Whole genome sequencing of polled Simmental and Holstein cows revealed a perfectly associated insertion/deletion variant (P_{202}ID) in Simmental and other beef breeds (Wiedemar *et al*., 2014). A total of 182 sequence variants have been identified as candidate mutations for polledness in Holstein cattle, including an 80 kb genomic duplication and three SNPs reported before (Wiedemar *et al*., 2014).

A majority of beef breeds have been identified to have the *Polled* locus in the same centromeric region of BTA 1, including in South Devon and Belgian Blue cattle. Georges *et al*., (1993) demonstrated that in a range of cattle breeds, including South Devon, a genetic linkage between the *Polled* locus and two microsatellite markers, GMPOLL-1 and GMPOLL-2,

and have assigned the corresponding linkage group to bovine chromosome 1. This is supported by Mariasegaram *et al*., (2012) demonstrating an association between taurine breeds (Angus, Hereford and Limousin) and composite taurine-zeba breeds (Brahman, Brangus, Droughtmaster and Santa Gertrudis). An association between a 303-bp allele and polled cattle was confirmed in these breeds; however, an additional allele (305 bp) was also associated but not fully predictive of polled cattle. Across this data, the microsatellite CSAFG29 was in sufficient linkage disequilibrium to the *Polled* allele in Australian Brahman cattle that it could potentially be used as a diagnostic marker in Brahman cattle, but this was not concluded in other breeds (Mariasegaram *et al.,* 2012). This suggests that the location of the *Polled* locus is similar in majority of cattle breeds. However, there could be breeds that diverge from this and multiple genes may be involved. This divergence is possibly related to where the breed originated from and diverged to (Allias-Bonnet *et al*., 2013).

There is the suggestion of other genes having an association with the development of horns in cattle, therefore potentially causing variation in the way the phenotype is expressed. Study of a differentially expressed group of annotated genes and loci within the mapped region on BTA 1 revealed a locus *(LOC100848215*), known in cow and buffalo only, which was highly expressed in the foetal tissue of wildtype horn buds compared to the tissue of polled foetuses (Wiedemar *et al*., 2014). This suggests that the presence of this long noncoding RNA is a prerequisite for horn bud formation. In addition, both transcripts associated with polledness in goat and sheep (*FOXL2* and *RXFP2*), have an overexpression in horn buds confirming their importance during horn development in cattle.

There are obvious variations between breeds with different accuracies found in currently available gene tests, however there is little evidence as to what this difference is. Current gene marker tests have been indirect, forming a haplotype (a set of characteristics on a single chromosome that are statistically associated), due to the complexity of locating a single marker that will be accurate across cattle breeds. This was described by Meat and Livestock in Australia, (2013) (See table 2). The Australian Gene Marker test reveals variable accuracies across breeds, suggesting breed variation is occurring.

2.10 **Limiting factors**

The gene pool in majority of cattle breeds is a major limiting factor, as to why genetic selection for polled cattle hasn't occurred in the past. This is largely evident in the dairy

industry in NZ and around the world. There has been a lack of selection for the polled phenotype, due to a lack of knowledge of the inheritance of phenotypes and that there are three possible genotypes. This leading to misconception, and speculation around the effects on production and reproduction by phenotypically selecting polled animals. However, today there is no evidence that the perceived reproductive losses are related to the polled gene (Milne, 1954).

Roy McGregor a Red and White Breeder states that "When I started in the industry (1982) there was little interest in Canada, I think mostly because there was a perception or stigma that polled genetics could not possibly be 100% pure" (Bloodlines, 2009, p.13). He goes on to say "his own ignorance of polled genetics had cost me twenty years" (Bloodlines, 2009, p.13). This supports the idea that people lacked information and knowledge of the mode of inheritance of polledness. This lack of knowledge was not just by breeders, also researchers. A complex single insertion-deletion event (P_{202}ID) was located and perfectly associated with the polled gene in most European cattle breeds in 2012 by Medugorac *et al*., (2012). However, due to the complexity of the *Polled* locus refining its location did not occur until 2014 (Weidemar *et al*., 2014).

Other challenges that have occurred are finding outcross genetics and informing people of polled genetics. Due to the small gene pool it has been a challenge to get polled genetics into the mainstream breeding programs of purebred and commercial herds, where polled cattle need to compete with current top genetics (Bloodlines, 2009). The wider adapted polledness is, the easier it will be to improve production and select for key production traits. There is obviously risk when single trait selecting, that it will negatively affect other key traits. This idea has been seen in the dairy industry with concern around cattle losing Breeding Worth (BW), this being stated by McPherson, LIC Breeding Manager (Holstein-Friesian New Zealand, 2012). However, while this would have been limiting previously, progress has now been made by LIC with the addition of the first polled Holstein-Friesian to the AI Sires list in 2012 (Holstein-Friesian New Zealand, 2012).

2.11 **Economic significance**

The damage of horns to other animals can cause financial losses, incurred due to trimming of bruised carcasses. According to the latest National Beef Audit, fewer than 6.4% of non-fed cattle and fewer than 8.6% fed cattle processed in Canada in 2010-11 had any type of horns,

and fewer than 3% had full horns, but the few horns that remained were costly (Beef Cattle Research Council, 2011). Processors lost $192,535 in 2011 ($0.06 per head) versus $106,003 ($0.032 per head) in 1999 due to extra labour costs for knocking off the horns (Beef Cattle Research Council, 2011), this increase in 2011 being due to increased cost of labour since 1999. The economic loss to the industry increases with increased bruising expected with the presence of horns, with the cost of bruised carcasses being estimated at $2.10 per head processed (Beef Cattle Research Council, 2011).

The cost of dehorning is not low, with potential additional cost including the requirement for pain relief to be administered prior to horn removal. The Dairy News (April, 2016) stated the cost for 200 replacements to be disbudded would be NZ$6-7 per head, which would end up costing NZ$1200-1400 for dehorning. This is similar to the model from The Cattle Site (June, 2014), where the dehorning cost was expected to average $11.79 per head (range from $5.84 to $22.89 per head), compared to the use of polled genetics, that would range from 47cents to $22.50 per head, with an average of $10.73 per head (The Cattle Site, June, 2014). Based on this model farmers could spend an additional $7.50 per head for polled genetics and break even with the average costs of dehorning. This model does not take into consideration the effects of reduced stress on calves or explicitly account for differences in the genetic potential of polled vs. horned sires.

Beyond the economic importance of removing horns there is a human significance. This being that removing horns by disbudding and dehorning is an unpleasant job, as stated by MacGregor “What is the one job done on a dairy farm that you would not do in front of a bus load of school children?” (Bloodlines, 2009, p.13). MacGregor goes on to say “the advantages of polled are obvious and you can see with your own eyes from the very first day a new polled calf is born, that the benefits of polled are real” (Bloodlines, 2009, p.13). This is an important aspect that needs to be considered when continuing with polled cattle. There are alternative options to removing horns that are both economically advantageous and less invasive.

2.12 **Conclusion**

The beef and dairy industries are economically valuable for New Zealand. Dairy is the largest goods export sector, averaging $14.4 billion of export revenue over the last five years. While beef is one of the most commonly exported meats from NZ, with meat and meat related

products exports worth $6.8 billion to the economy. Livestock production in NZ is constantly being reviewed to meet ever changing demands and animal welfare regulations.

The value of horned cattle in the modern beef and dairy production systems is now being questioned. While horns are common in many beef and dairy cattle breeds, they pose animal welfare, animal health and human/farmer health and safety concerns. The current management practises widely used for removal of horns are disbudding in the early stages of life or dehorning later. This was prior to the evidence supporting the location of the *Polled* locus. Recently, the location of the *Polled* locus has been narrowed down to chromosome 1 (BTA1) in *Bos taurus*. A single perfectly associated insertion/deletion variant (P_{202}ID) in Simmental and other beef cattle has been found. However, with this advancement in the location of the polled gene, indirect gene marker tests have been developed in Australia, using haplotypes to identify the presence of the polled gene. These gene tests are unavailable in NZ, so there is room in the market for a gene test identifying horned and polled cattle to be commercialised in NZ, as the benefits that come with polled cattle are clear, both in the dairy and beef industries. This includes economic savings due to reduced labour (both on farm and at slaughter), the improved health and well-being of calves through reduced stress (therefore no setbacks) and hornless cattle remove any speculation involving animal welfare issues.

Chapter 3

Materials and Method

3.1 Ethical statement

No formal ethical approval was required; some DNA samples were stocked in the laboratory from previous study. The remaining DNA samples were obtained by routine sampling, with consent from all cattle owners.

3.2 Cattle investigated and Blood sampling

A total of 876 individual blood samples were collected, of which 467 were genotyped to validate the gene test. These 876 animals originated from five British cattle breeds belonging to the *Bos taurus* subspecies.

Sampling involved small cuts being made in the ear of each individual animal using cutters. Blood was collected directly onto FTA paper, 1.2mm punches were taken and purified from the FTA paper for DNA analysis. This was then genotyped in the Gene Marker Laboratory based on the Lincoln University campus. Each sample had an animal identification number recorded, including the birth year. All animals sampled and genotyped in this study are presented in Appendix A (A1-A5).

Three hundred and seventy-five Belgium Blue cattle were sampled from Lockwood Smith (Woodleigh Stud) located in Northland (Farm B), of which one hundred and fifty-three were used in the gene test. Thirty-four South Devon cattle were sampled from Peter Foss located in Aria, Te Kuiti (Farm A). Thirty-six South Devon cattle were sampled from Richard Van Asch (Aschwood Stud) located in Blenheim (Farm C). One hundred and eighty-seven Hereford cattle were sampled from Collin Gibsons (Seadowns Stud) located in Omarau (Farm D), of which ninety animals were gene tested. Two-hundred and sixty-seven Hereford cattle were sampled from Rob Stokes (Richon Stud) and Rob Burrows (Beechwood Stud) located in the Lee's Valley (Oxford) (Farm E), of which twenty-six were used in for gene testing. Seventy-four milking Shorthorn-cross calves (born 2017) were sampled from Phil Garrett located in Springston (Farm F) and forty- four Hereford cattle were sampled from Greg Chamberlain (Capethorne Stud) located in Cheviot (Farm G). Fifty-one cattle including Holstein-Friesian

(horned) and dairy x beef cross (Hereford) were sampled from Keith Flay located in Rangiora (Farm H). Of these, four were gene tested. One hundred Holstein-Friesian calves (2016 born) were sampled from John Greenslade located in Lincoln (Farm I).

3.3 Phenotypes and Declaration of the underlying *POLLED* Genotype

Phenotypic records were sourced from Performance Beef Breeders (PBB) NZ Limited for all Hereford cattle. Shorthorn records were sourced from MINDA and all other phenotypic records were sourced directly from the cattle owners.

Polledness or the complete absence of horns is a visible phenotype that can be identified at a relatively young age (four to six months). Because the growth of scurs occurs later in life than horns, phenotyping for some scurred animals will not be possible until nine to eighteen months of ages (Capitan *et al*., 2009). Polled animals are declared as polled (name suffix 'B') in their pedigree certificate and/or other records. One B designates polled animals with the BB or AB genotype underlying the *Polled* locus. Genotypes presenting BB are declared homozygous polled. Animals with an AA genotype are declared homozygous horned (name suffix A). Due to polled being dominant, cattle presenting as AB (heterozygous polled) will phenotypically be polled, but could produce horned offspring if mated with another heterozygous polled animal.

3.4 PCR primers and PCR amplification

PCR amplification and SSCP analysis was completed in the Gene Marker laboratory at Lincoln University, (undertaken by Dr Huitong Zhou).

Two PCR primers, TCAAGAAGGCGGCACTATCT and CAAAGGCAGAGATGTTGGTC, were designed to amplify a gene region containing a 202 bp InDel (referred to as P_{202}ID) located between IFNAR2 and OLIG1. These primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

PCR amplification was performed in a 15-µL reaction containing the genomic DNA on a 1.2-mm punch of the FTA paper, 0.25 µM primers, 150 µM dNTPs (Bioline, London, UK), 2.5 mM Mg^{2+} , 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× reaction buffer supplied with the enzyme.

The thermal profile consisted of 2 minutes (min) at 94 °C, followed by 35 cycles of 30 seconds (s) at 94 °C, 30 s at 60 °C and 30 s at 72 °C, with a final extension of 5 min at 72 °C. Amplification was carried out in S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA).

3.5 **Polymorphism screening and sequencing of allelic variants**

PCR amplicons were screened for sequence variation using SSCP analysis. 0.7-µL of each amplicon was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 95 ^{o}C for 5 min, the samples were rapidly cooled on wet ice and then loaded on 16 cm × 18 cm, 14% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels.

Electrophoresis was performed using Protean II xi cells (Bio-Rad) in 0.5× TBE buffer at 10 ^{o}C at 250 v for 18 h. The gels were silver-stained according to the method of Byun et al. (2009).

3.6 **Data Analysis**

Data collected from the gene test was visually assessed and the phenotypic data and genotypic data compared. The results are presented below in tables for each farm, giving the accuracy of determination of the phenotype for each farm and breed sampled.

Chapter 4

Results

After amplifying a gene region containing a 202 bp InDel (referred to as P_{202}ID) located between IFNAR2 and OLIG1. Three banding patterns (AB, AA and BB) were observed, two of which are illustrated in Figure 3. This includes two banding patterns signifying two variants, A (polled gene present) and B (horn gene present) and come in either heterozygous or homozygous forms.

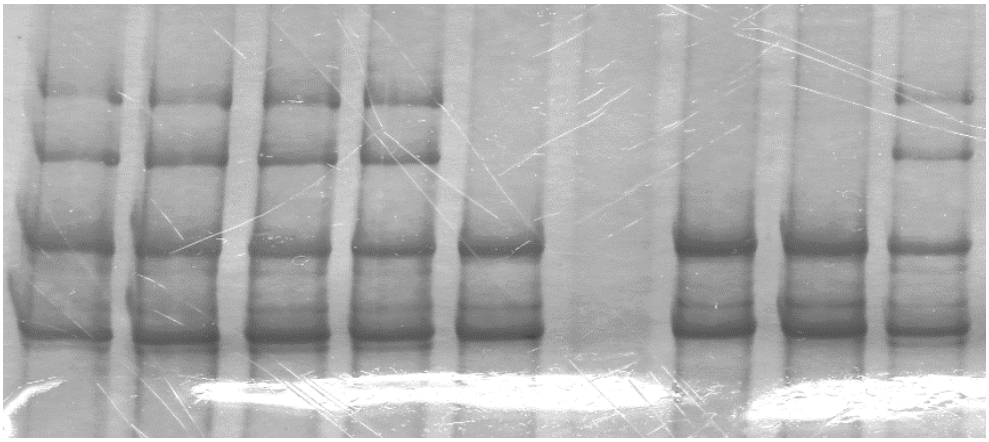

Figure 3: PCR-SSCP of DNA samples.

4.1 Farm Differences

Table 3: Farm A, South Devon (n=40[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	16		
Total	16	16	100%
Polled			
AB	18		
BB	0		
Total	18	18	100%

No PCR result

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

Table 3 reveals that of the 34 South Devon Cattle tested 16 were genotyped horned, 18 were heterozygous polled and no cattle were homozygous polled. When comparing this to the phenotype expressed by animals, 16 cattle were horned and 18 cattle were polled. Thus, the genotype produced by the gene test matched the phenotype with 100% accuracy.

Table 4: Farm B, Belgium Blue (n=375[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	4		
Total	4	0	0%
Polled			
AB	67		
BB	42		
Total	109	113	96.5%

No phenotype available or PCR result

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

Table 4 reveals the results of 113 Belgium Blue cattle that were put through the DNA test, that had recorded phenotypic data. There was a remaining 46 animals that were put through the gene test that did not have phenotypic data, therefore an accuracy could not be made for these 46 cattle.

Based from the 113 animals, table 4 reveals that four animals were genotyped to be homozygous horned, 67 were genotyped to be heterozygous polled and 42 animals were homozygous polled. Based on the recorded phenotypic data all 113 animals expressed the polled phenotype. Thus, the test matched the genotype and phenotype with 96.5% accuracy.

Table 5: Farm C, South Devon (n=38[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	17		
Total	17	17	100%
Polled			
AB	12		
BB	7		
Total	19	19	100%

No PCR result

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

Table 5 reveals that of the 38 South Devon cattle sampled, two cattle presented no PCR result, 17 were genotyped to be homozygous horned, 12 were heterozygous polled and seven were homozygous polled. Therefore, of the animals that gave a result the gene test was 100% accurate, with 17 South Devon phenotyped to be horned and 19 South Devon phenotype to be polled.

Table 6: Farm F, Shorthorn (n=75[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	48		
Total	48	48	100%
Polled			
AB	22		
BB	4		
Total	26	26	100%

No PCR result

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

Table 6 reveals that of the 75 Short Horn Cattle tested, one animal did not give a PCR result, 48 animals presented a homozygous horned genotype, 22 animals were heterozygous polled and four animals were homozygous polled. This matches to 100% of the phenotypic data supplied, with 48 animals expressing horns and 26 expressing the polled phenotype.

Table 7: Farm D, Herefords (n=106[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	69		
Total	69	51	73.9%
Polled			
AB	18		
BB	2		
Total	20	38	52.6%

No PCR run

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

Table 7 reveals the split of the 90 Hereford cattle that were put through the PCR, across three phenotypes (horned, polled and scurred). There were 51 animals that expressed to be horned and 38 that were recorded as polled. Along with this there was one animal that had been identified as scurred, but was still tested and presented a genotype of AA (homozygous horned). The scurred condition involves a separate gene, thus this is unlikely to be a fair representation of the genotype of this individual. The scurred individual was not included in either the polled or horned phenotype category. However, table 7 does reveal that there was an accuracy of 63.3%. With 69 animals being genotyped to be homozygous horned, 18 being genotyped as heterozygous polled and two being homozygous polled.

Table 8: Farm E, Hereford (n=44[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	11		
Total	11	7	63.6%
Polled			
AB	7		
BB	8		
Total	15	19	78.9%

No PCR run

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

The results from table 8 reveal that of the 26 Hereford used in the PCR, eleven cattle were genotyped to be homozygous horned, seven were heterozygous polled and eight were homozygous polled. The results then go on to show that only seven were phenotypically recorded as horned and 19 were recorded as polled. Thus, the accuracy between the phenotypic and genotypic data was 71.5%.

Table 9: Farm G, Hereford (n=46[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	2		
Total	2	0	0%
Polled			
AB	22		
BB	20		
Total	42	44	95.5%

No PCR result

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

The results from table 9 reveal that of the 44 Hereford Cattle tested two showed the horned genotype, 22 showed to be heterozygous polled and 20 cattle were homozygous polled. When comparing this to the phenotype expressed by animals, no cattle were horned and 44 cattle were polled. Thus, the genotype produced by the gene test matched the phenotype with an accuracy of 95%.

Table 10: Farm H, Holstein-Friesian and beef cross (n=52[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	2		
Total	2	2	100%
Polled			
AB	2		
BB			
Total	2	2	100%

No PCR run

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

The results from table 10 reveals that of the four Holstein-Friesian cattle were used in the research out of 52 cattle sampled. Two animals were pure Holstein-Friesian and two were Holstein-Friesian x Hereford-cross. Based on the phenotypic data, two animals were polled and two had recorded phenotypes as being polled (Cross-bred cattle). The genotypes found matched 100% with the phenotypes recorded. Two cattle were homozygous horned and two animals were recorded as being heterozygous polled.

4.2 **Breed differences**

Table 11: Hereford Accuracy (n=196[#])

	No. with genotype	No. with Phenotype	Accuracy
Horned			
AA*	82		
Total	82	58	70.7%
Polled			
AB	47		
BB	30		
Total	77	101	76.2%

No phenotype or PCR result or no PCR run

* AA= Homozygous horned, AB= Heterozygous polled (polled gene is dominant), BB= Homozygous polled.

It is clear that the results reveal that a high level of confidence can be held when using the gene test in breeds such as South Devon (100%), Shorthorn (100%) and Belgian Blue (96.5%). However, table 11 reveals a lower average accuracy of 73.5% across Hereford cattle from varying locations. It is clear there is potential farm or breed variation occurring.

The Holstein-Friesian cattle that were sampled included two fully horned Holstein-Friesians and two Holstein-Friesian x Hereford cross-bred steers. This was 100% accurate but is not a fair representation as no polled Holstein-Friesian cattle were tested and thus reliability was restricted by sample size. However, the gene test was able pick up 100% of the homozygous horned animals.

The results indicate that overall the gene test was accurate at finding the genotype status of the selected cattle breeds, matching the phenotypic data and identifying heterozygous polled animals (AB, carriers of the horn gene). The exception was the Hereford cattle, where the accuracy is lower than for all other breeds.

Chapter 5

Discussion

The use of gene tests and gene-marker tests are similar to the use of estimated breeding values (eBVs), where EBVs estimate all the genetic variation, and the specific sources of variation (genes) are unknown. However, gene marker tests and gene tests reveal the genotype of an animal for specific DNA markers for a particular trait, but do not account for all of the genetic variation that might be possible as a consequence of the activity of other genes.

The use of DNA marker information can allow for early prediction of the genetic merit of an animal before phenotypic records are collected, thus increasing the accuracy for the selection of young sires and decreasing generation interval. Scott Hansen, former Meat and Livestock Australia CEO stated that: “using normal breeding practises it would take 39 years, but with the new Gene Poll (current gene test available in Australia) gene-marker test it reduces that down to 8 years” (ABC News, 2014).

In this investigation, compelling results were revealed in South Devon, Belgian Blue and Shorthorn cattle, where high prediction accuracies were observed. However, the accuracy of the gene test used declined in Hereford and Holstein-Friesian cattle, reducing the confidence in the utility of the test in those breeds.

A possible cause of variation is through ‘human error’, leading to the miss-recording of phenotype and parentage. There has been extensive research conducted to understand the true nature of ‘human error’ (Hollnagel, 2014). However, the results have been contradictory. One review concluded that erroneous actions were unavoidable consequences of the imperfection of humans, and that they will still occur under the best conditions. Erroneous actions were by their nature unpredictable in both form and frequency, although some patterns could be found (Hollnagel, 2014). Another review determined that erroneous actions were results of unfavourable working conditions or an unforgiving environment. Erroneous actions were predictable both in form and frequency and it was therefore possible in principle as well as in practise to significantly reduce their number (Hollnagel, 2014). According to this view the best resolution is to improve the

working conditions by providing humans with appropriate tools and by amplifying human strengths rather than reducing human weaknesses.

Although genotyping errors affect most data and could therefore markedly influence the biological conclusions drawn from a study, they are often neglected or unidentified. Errors have various causes, but their occurrence and effect can be limited by considering possible causes in the production and analysis of the data. Pompanon et al., (2005) proposed a protocol for estimating error rates and recommend that these measures be systemically reported to attest the reliability of published genotyping studies. Therefore, this may need to be considered to further validate the gene test used here.

5.1 Farm to farm variation

Of the samples that were collected not all were used in the present study, as a clear pattern emerged in the various breeds. As a result a large majority of the Holstein-Friesian cattle were not typed. This was because all the Holstein-Friesian cattle had the horned phenotype, with the exception of two Holstein-Friesian x Hereford-cross steers. These two animals were given the polled phenotype and were genotyped to be heterozygous polled (AB). This could show an underlying relationship between Hereford cattle and Holstein-Friesian cattle, while also demonstrating the potential for the polled gene to be effectively introduced into Holstein-Friesian herds. This could reduce the use of disbudding and advancing dairy farming systems before regulations and restrictions are implemented. Even though there has been a separate gene located on BTA 1 specifically associated with the polled genotype in Holstein-Friesian cattle (*Holstein* mutation), this overlaps the *Polled* locus seen in most beef breeds (Wiedemar et al., 2014). Therefore, there could be potential for the gene test to be used in cross-bred cattle.

The results from the Hereford cattle were inconsistent across farms, ranging from 63.3 % on farm D, to 71.3% farm E and 95.5% on farm G. This is best explained by miss recording of phenotypic data and miss-recording of parentage, effecting the accuracy of the results. The lower accuracies on Farm D and Farm E, could be explained by both horned and polled cattle being on the same property and kept in mixed herds, whereas Farm G solely carried polled cattle. Having mixed herds may increase the likelihood of miss-recording phenotypes. Miss-recording may also be influenced by having different people identifying calf phenotypes. For

example on Farm G, the owner is the sole person identifying calf phenotype and parentage, and within 24 hours of birth.

The time at which the phenotype was collected could also cause miss recording of phenotypes. The presence of horns in young stock is harder to identify, as the horn does not attach to the skull until approximately 8 weeks of age (Dairy NZ, n.d), recording accuracy may vary. The rate of horn development may also vary within and across breeds. Genotyping animals using the gene test would allow the ability to determine potential animals that are likely to have horned offspring, leading to improved sire selection.

There is the suggestion of other genes being associated with the development of horns in cattle, potentially causing variation in the way the phenotype is expressed. Wiedemar et al., (2014) studied the differential expression of the annotated genes and loci within the mapped region on BTA 1, revealing a locus *(LOC100848215*), known in cow and buffalo only, which is more highly expressed in foetal tissue of wildtype horn buds compared to tissue of polled foetuses. This implies that the presence of this long noncoding RNA is a prerequisite for horn bud formation. In addition, both transcripts associated with polledness in goat and sheep (*FOXL2* and *RXFP2*), show an overexpression in horn buds, confirming their importance during horn development in cattle.

There is also the potential for miss-recording of calves to dams, this effecting the recording of pedigree. Pedigree has been used to determine animals phenotypes for horned and polled historically, but it frequently does not include the genotype of individuals or assist to identify animals that carry both horned and polled alleles (AB in this study). Miss-recording of parentage could occur as cattle herds are not commonly DNA tested to check parentage, especially in beef herds. Recording error could therefore play an important role in the miss-match between phenotype and genotype observed in this research. It has been shown in dairy farming systems there is a high chance of miss-recording of parentage, the Lincoln University Demonstration Farm of 650 cows had 192 of their 2010-born calves parentage tested by LIC, and the results showed a 31% miss-recording rate at birth (GeneMark DNA Parentage Verification, n.d). However, this would be expected to be lower in beef herds as herd sizes are generally smaller and there are many small holdings. Fifty-five percent of beef producers have less than 50 beef cattle, with only 7% of farms having over 500 beef cattle (Beef Industry Overview, 2017). This compares to the average dairy herd in NZ being 419

cows in 2015/16 (National dairy statistics, 2016). The larger beef herds would be expected to be in extensive farming systems and thus may have higher levels of miss-recording.

5.2 Breed variation

5.2.1 South Devon and Belgian Blue

It is clear that the gene test was able to successfully identify homozygous and heterozygous polled South Devon with 100% accuracy. The results found that the test was accurate for Belgian Blue, with 96.5% accuracy. This could be influenced by a slight breed variation of the polled gene or more likely phenotype recording errors. An accuracy of 96-100% gives great confidence that the test should remain accurate when used across different farms. The concept of possible farm to farm variation could not be tested though, as only one Belgian Blue and two South Devon farms (both 100% accurate) were used.

A majority of beef breeds have been previously identified to have the *Polled* locus in the same position of BTA 1, including in South Devon and Belgian Blue cattle. This is supported by Georges *et al*., (1993) demonstrating in a range of cattle breeds, including South Devon, a genetic linkage between the *Polled* locus and two microsatellite markers, GMPOLL-1 and GMPOLL-2, and has assigned the corresponding linkage group to BTA 1. This suggests that the location of the *Polled* locus is similar in the majority of beef breeds, but there could be breeds that diverge from this. This divergence is possibly related to where the breed originated and has since diverged to (Allias-Bonnet *et al*., 2013).

There are no gene tests currently available that have been proven to identify polled and horned South Devon and Belgian Blue cattle. This test would provide the beef industry with a key tool to further advance these breeds.

5.2.2 Shorthorn

In the present study the accuracy of the gene test within the Shorthorn cattle tested was 100%. Therefore, it could be assumed that the current gene test is accurate at identifying the heterozygous polled and homozygous polled and homozygous horned individuals. Similar results have been found for the Poll Gene marker test from Australia showing a 94% accuracy (Meat and Livestock Australia, 2013).

The Shorthorn cattle used in this research were genetically based on known Shorthorn lines, but some were cross-bred with Jersey, Ayrshire and other red dairy breeds of cattle (including red Holstein-Friesian). This could have had an effect on the accuracy of the results. This gene test has revealed potential to work across a range of breeds, due to the high accuracy in cross-bred Shorthorns.

Based on the Shorthorn results it is clear that the test can accurately identify polled and horned individuals and would be assumed to remain this accurate when extended into a larger population.

5.2.3 Hereford

The Hereford results revealed an inconsistency, with the accuracy ranging from 63.3 % (Farm D), through 71.3% (Farm E), to 95.5% (Farm G), with a breed average of 76.7% accuracy. This contrasts results for the Australian Poll Gene marker test, which has an accuracy of 96% in Hereford cattle (Meat and Livestock Australia, 2013). The most likely explanation for this variation is between-farm variation, caused by the miss-recording of phenotypes. This miss-recording would likely be due to miss-identification of horned cattle at the young ages and the inaccurate recording of parentage.

When investigating further into the inaccurate Hereford results from one property (Farm G), there was the potential for the two cattle that were genotyped to be horned (AA in this test) to have ancestry involving horned cattle, going back more than two generations. However, their dams and sires were polled, and thus one might have expected a polled animal. This variation could have occurred due to bulls being recorded as polled but possibly being heterozygous polled, carrying one horned allele that was inherited by the off spring. The gene test used here would allow identification of heterozygous polled (carrier) animals, helping to reduce the likelihood of horned offspring being produced. Only homozygous polled bulls could then be used in breeding programmes to reduce the likelihood of producing horned offspring. This would improve the accuracy of breeding decisions.

Therefore, based on current research there is little evidence available to explain the decline in accuracy in Hereford cattle, other than through human error (miss-recording).

5.2.4 Holstein-Friesian

There are well known differences in the genes involved with the polled genotype in Holstein-Friesian cattle. Significant amounts of research has been carried out in various breeds of cattle with the *Polled* locus being located on BTA 1 in British beef breeds. The area in which breed variation is likely to occur is the area along BTA 1 where the polled mutation occurs.

High-density SNP genotyping is used to identify different polled associated haplotypes co-localized on BTA 1. The Simmental (and other beef breeds) *Polled* locus has been refined to a 212 kb region and an overlapping region of 932 kb has been identified that contains the Holstein-Friesian *Polled* mutation (Wiedemar et al., 2014). Subsequently, whole genome sequencing of the polled Holstein-Friesian cows was used to determine polled-associated genomic variants, with a total of 182 sequence variants identified as candidate mutations for polledness in Holstein-Friesian cattle, including an 80 kb genomic duplication and three SNPs reported previously by Wiedemar et al., (2014). The Holstein-Friesian *Polled* mutation is likely have an effect on the results from the gene test, possibly causing breed variation, due this mutation only being present in Holstein-Friesian cattle. Therefore may reduce the reliability of the test if polled Holstein-Friesian cattle were tested.

There has been a possible association occurring between Holstein-Friesian and Hereford cattle. This is shown by two cross-bred cattle (Hereford x Holstein-Friesian) that were tested and that presented accurate results upon testing. This indicates that there is a possible linkage within this cross, where the test can effectively determine heterozygous polled and homozygous polled animals.

5.3 Confounding effects

The main confounding effect that could be influencing the results presented here is the presence of scurs. Scurs have a different set of genes effecting there development (White & Ibsen, 1936; Long & Gregory, 1978) and they are present when an animal is either horned or polled, but are only visible if an animal is polled. There was one Hereford that was identified to be scurred based on phenotype and presented to be homozygous horned (AA). It is unsure how the scurred gene interacts with the genotype that is detected with this gene test, and thus more research should be conducted in this area to gain an understanding of the effect scurs has on the results. There are different genes involved in regards to scurs and

the effect they have on horn development is unknown (Long & Gregory, 1978; Capitan *et al*., 2009).

Scurs could also have a negative effect when phenotyping calves at a young age, as age has an effect on the time at which scurs become present, as was demonstrated by a skull dissection made by Brenneman *et al*., (1996). This mode of inheritance and the expression of phenotype being influenced by age of the animal, complicates any form of study of the inheritance of phenotypes. Thus, a definitive DNA test for differentiating scurred, horned and polled animals is required to make successful breeding decisions.

5.4 **Previous Gene tests identifying polled and horned status**

The Australian Poll Gene Marker test (released in 2010) is used to measure the likelihood that a polled animal only carries the polled gene. Originally the test was based on a single gene marker and has been refined to include a further nine markers. In some breeds, such as Brahman, a single allele at the DNA marker was almost always associated with polledness and other alleles always associated with horned, making the test highly accurate in these breeds. However, in other breeds, multiple alleles have associations with both polledness and horned, so the test could not accurately determine between homozygous polled and heterozygous polled (Meat and Livestock Australia, 2013). This supports the association of multiple alleles to determine horned status, while also supporting the idea of across breed differences.

When comparing against the accuracy of the Poll Gene test (Meat and Livestock Australia, 2013) it is clear there are similarities. The current study found Herefords to have an average accuracy of 76.7% compared to 96% in the Poll Gene test (Meat and Livestock Australia, 2013). While the Shorthorn results were more accurate in the current study, with 100% accuracy compared to 94% (Meat and Livestock Australia, 2013). Based on these accuracies it can be assumed that there are breed variations between Hereford and Shorthorn cattle, however it is not clear what causes this variation. One hypothesis may be that these differences occurred far back in the animals ancestry, when herds diverged from their country of origin. Divergence may have caused different varieties of Hereford and Shorthorn cattle being produced in different countries, such as Australia and NZ, this could explain the variation in accuracy between the Poll Gene test and this test results.

Currently, no gene test is able to identify polled genotypes in Holstein-Friesian cattle. Therefore, this could be a potential target area in the market and where the uptake from farmers would be high due to regulations potentially brought in that restricted the use of dehorning practises.

5.5 **Further research**

The exact location of a polled gene has been determined in some breeds, but little is known about the associated genes and mutations that interact with the polled gene. Therefore, there is the potential for breed differences occurring at the genome level and this cannot be determined until further research is conducted.

It is clear that there is a need for further research to distinguish the potential breed differences that could cause the fluctuation in the accuracy of the gene test across breeds. This research would need to determine if there are breed differences across beef breeds and between beef and dairy breeds. Further research is also required to determine the potential for association between the *Polled* locus and *scurs* locus. This would eliminate any potential confounding effects from the presence of the *scurs* locus.

Further validation for the gene test used here would be required across a large variety of breeds, including a larger number of animals in each breed. To commercialise the test successfully and to get a high farmer uptake would require involvement from breed associations and the involvement of companies that sell genetics, both beef and dairy. More focus could be directed to the dairy industry as the beef industry does have a gene pool of currently available polled animals, compared to the dairy industry with a very limited polled gene pool of sires with high genetic merit. With further validation it would be expected that the accuracy of the gene test in each breed would improve or stay similar to what has been found in this research. An increased use of DNA-based parentage testing would lead to improved accuracy of parentage recording, eliminating some of the human error (miss-recording of phenotype and parentage).

The rate of farmer uptake would also have to be determined. This could be achieved through surveying a randomly selected group of farmers, determining if they would find this test useful if commercialised. To improve farmer uptake, it would involve a commitment from

breed associations (e.g. NZ Hereford Association) and genetics companies to lead the way with promoting the benefits of polled cattle. New technology does come with a cost, careful economic analysis must be performed prior to implementing any new technology for selection or management purposes to determine if the end result justifies the cost.

Chapter 6

Conclusions

It can be concluded that compelling results were shown in South Devon, Belgian Blue and Shorthorn cattle tested. However, accuracies declined for Hereford and Holstein-Friesian cattle, reducing the confidence there is for accurate results being produced. Holstein-Friesian cattle were limited as little variation was shown, due to very few polled animals being available and a small sample size. To increase the accuracy across all breeds, a larger group of animals needs to be tested, across a wider range of breeds.

The main factors that are affecting the results are probably human errors in recording phenotype. There could be breed variation in test accuracy, but the accuracy for Hereford cattle was not low across all farms and was varied, farm to farm variation in phenotyping is more likely to be the determining factor. This primarily comes down to possible human error of miss-recording phenotype and parentage.

There is the possibility that scurs were having a confounding effect on the results. As the presence of the scurs gene is only identifiable at a later stage of life so may not have been identified, due to phenotypes generally being recorded at the early stages of life. Scurs can only be visually assessed when an animal is polled, as horns are at the same location as scurs and act as a mask. However, the confounding effect could not be determined and requires further research to understand the interaction between the *Polled* locus and *scurs* locus.

It has been commonly perceived that there are production and conformation losses in polled cattle. However, there is more evidence suggesting no differences in production between horned and polled cattle and that the differences that have occurred over time is due to the gene pool size and the selection processes that have occurred. For example poor selection occurring due to polled genetics being favourable therefore, reduced selection pressure likely occurring and poorer animals being kept. This could reduce the performance of the polled herd, whilst only the highest producing horned cattle would have been kept. Hence producers and scientists need to recognise breeding for polled cattle as an alternative non-invasive method of dehorning and could be made easier by the adoption of using gene tests to help make the selection process more accurate and time efficient.

It is hard to create a test with 100% accuracy across all breeds of cattle. An accuracy of 90% or above is still positive and will enable the cattle industry to move forward and eliminate horns. Even with the potential for an animal to be missed, horned cattle numbers will decrease, thus reducing the use of management practises such as disbudding.

It is important to know the homozygous and heterozygous state of cattle to effectively reduce the proportion of horn alleles in a breeding population while monitoring the masking of the scurred phenotype. Propagation of the polled gene in purebred herds is inhibited due to the inability to distinguish what animals are heterozygous or homozygous. Genetic testing would be advantageous and this test could be utilised to significantly assist the beef and dairy industries to develop farming systems that will meet best practise animal welfare conditions.

Appendix A

Cattle Records

A.1 South Devon Raw Data

Table A1: South Devon Raw data, from Farm A (n=34)

Tag	Breed	Phenotype	PC region	Mismatch
11007	South Devon	Horned	AA	
11025	South Devon	Horned	AA	
11032	South Devon	Horned	AA	
11069	South Devon	Horned	AA	
11084	South Devon	Horned	AA	
11091	South Devon	Horned	AA	
11096	South Devon	Horned	AA	
11184	South Devon	Horned	AA	
11187	South Devon	Horned	AA	
12004	South Devon	Horned	AA	
12005	South Devon	Horned	AA	
12022	South Devon	Horned	AA	
12069	South Devon	Horned	AA	
12074	South Devon	Horned	AA	
12092	South Devon	Horned	AA	
12159	South Devon	Horned	AA	
11001	South Devon	Polled	AB	
11008	South Devon	Polled	AB	
11106	South Devon	Polled	AB	
11114	South Devon	Polled	AB	
11120	South Devon	Polled	AB	
11134	South Devon	Polled	AB	
11166	South Devon	Polled	AB	
12016	South Devon	Polled	AB	
12026	South Devon	Polled	AB	
12099	South Devon	Polled	AB	
12106	South Devon	Polled	AB	
12114	South Devon	Polled	AB	
12136	South Devon	Polled	AB	
12146	South Devon	Polled	AB	
12149	South Devon	Polled	AB	
12165	South Devon	Polled	AB	
12182	South Devon	Polled	AB	
12187	South Devon	Polled	AB	

Table A2: South Devon Raw data, from Farm C (n=36)

Tag	Breed	Phenotype	PC region	Mismatch
215	South Devon	Horned	AA	
416	South Devon	Horned	AA	
617	South Devon	Horned	AA	
724	South Devon	Horned	AA	
921	South Devon	Horned	AA	
925	South Devon	Horned	AA	
928	South Devon	Horned	AA	
1141	South Devon	Horned	AA	
1420	South Devon	Horned	AA	
1423	South Devon	Horned	AA	
1424	South Devon	Horned	AA	
1428	South Devon	Horned	AA	
1429	South Devon	Horned	AA	
1435	South Devon	Horned	AA	
1439	South Devon	Horned	AA	
14203	South Devon	Horned	AA	
MTR15	South Devon	Horned	AA	
828	South Devon	Polled	AB	
1149	South Devon	Polled	AB	
1330	South Devon	Polled	AB	
1346	South Devon	Polled	AB	
1425	South Devon	Polled	AB	
1432	South Devon	Polled	AB	
1433	South Devon	Polled	AB	
1434	South Devon	Polled	AB	
1437	South Devon	Polled	AB	
1438	South Devon	Polled	AB	
1441	South Devon	Polled	AB	
13102	South Devon	Polled	AB	
1421	South Devon	Polled	BB	
1422	South Devon	Polled	BB	
1427	South Devon	Polled	BB	
1430	South Devon	Polled	BB	
1431	South Devon	Polled	BB	
1442	South Devon	Polled	BB	
1443	South Devon	Polled	BB	

A.2 Belgian Blue Raw Data

Table A3: Belgian Blue Raw Data, from Farm B (n=113)

Tag	Breed	Phenotype	PC region	Mismatch
527	Belgium Blue	Polled	AA	*
586	Belgium Blue	Polled	AA	*
1550	Belgium Blue	Polled	AA	*
1583	Belgium Blue	Polled	AA	*
503	Belgium Blue	Polled	AB	
505	Belgium Blue	Polled	AB	
513	Belgium Blue	Polled	AB	
523	Belgium Blue	Polled	AB	
524	Belgium Blue	Polled	AB	
525	Belgium Blue	Polled	AB	
536	Belgium Blue	Polled	AB	
536	Belgium Blue	Polled	AB	
542	Belgium Blue	Polled	AB	
543	Belgium Blue	Polled	AB	
544	Belgium Blue	Polled	AB	
547	Belgium Blue	Polled	AB	
548	Belgium Blue	Polled	AB	
549	Belgium Blue	Polled	AB	
550	Belgium Blue	Polled	AB	
554	Belgium Blue	Polled	AB	
557	Belgium Blue	Polled	AB	
563	Belgium Blue	Polled	AB	
564	Belgium Blue	Polled	AB	
568	Belgium Blue	Polled	AB	
569	Belgium Blue	Polled	AB	
572	Belgium Blue	Polled	AB	
573	Belgium Blue	Polled	AB	
578	Belgium Blue	Polled	AB	
579	Belgium Blue	Polled	AB	
588	Belgium Blue	Polled	AB	
590	Belgium Blue	Polled	AB	
592	Belgium Blue	Polled	AB	
594	Belgium Blue	Polled	AB	
599	Belgium Blue	Polled	AB	
600	Belgium Blue	Polled	AB	
1501	Belgium Blue	Polled	AB	
1502	Belgium Blue	Polled	AB	
1503	Belgium Blue	Polled	AB	
1504	Belgium Blue	Polled	AB	
1509	Belgium Blue	Polled	AB	
1512	Belgium Blue	Polled	AB	
1515	Belgium Blue	Polled	AB	

1524	Belgium Blue	Polled	AB	
1526	Belgium Blue	Polled	AB	
1528	Belgium Blue	Polled	AB	
1533	Belgium Blue	Polled	AB	
1537	Belgium Blue	Polled	AB	
1541	Belgium Blue	Polled	AB	
1542	Belgium Blue	Polled	AB	
1543	Belgium Blue	Polled	AB	
1545	Belgium Blue	Polled	AB	
1547	Belgium Blue	Polled	AB	
1549	Belgium Blue	Polled	AB	
1551	Belgium Blue	Polled	AB	
1553	Belgium Blue	Polled	AB	
1555	Belgium Blue	Polled	AB	
1556	Belgium Blue	Polled	AB	
1558	Belgium Blue	Polled	AB	
1559	Belgium Blue	Polled	AB	
1563	Belgium Blue	Polled	AB	
1571	Belgium Blue	Polled	AB	
1576	Belgium Blue	Polled	AB	
1578	Belgium Blue	Polled	AB	
1579	Belgium Blue	Polled	AB	
1580	Belgium Blue	Polled	AB	
530	Belgium Blue	Polled	AB	
551	Belgium Blue	Polled	AB	
556	Belgium Blue	Polled	AB	
1513	Belgium Blue	Polled	AB	
1522	Belgium Blue	Polled	AB	
1568	Belgium Blue	Polled	AB	
502	Belgium Blue	Polled	BB	
504	Belgium Blue	Polled	BB	
512	Belgium Blue	Polled	BB	
515	Belgium Blue	Polled	BB	
535	Belgium Blue	Polled	BB	
541	Belgium Blue	Polled	BB	
545	Belgium Blue	Polled	BB	
552	Belgium Blue	Polled	BB	
561	Belgium Blue	Polled	BB	
562	Belgium Blue	Polled	BB	
565	Belgium Blue	Polled	BB	
577	Belgium Blue	Polled	BB	
582	Belgium Blue	Polled	BB	
589	Belgium Blue	Polled	BB	
591	Belgium Blue	Polled	BB	
601	Belgium Blue	Polled	BB	
604	Belgium Blue	Polled	BB	
1507	Belgium Blue	Polled	BB	

1508	Belgium Blue	Polled	BB	
1510	Belgium Blue	Polled	BB	
1514	Belgium Blue	Polled	BB	
1517	Belgium Blue	Polled	BB	
1518	Belgium Blue	Polled	BB	
1521	Belgium Blue	Polled	BB	
1527	Belgium Blue	Polled	BB	
1529	Belgium Blue	Polled	BB	
1531	Belgium Blue	Polled	BB	
1534	Belgium Blue	Polled	BB	
1539	Belgium Blue	Polled	BB	
1540	Belgium Blue	Polled	BB	
1544	Belgium Blue	Polled	BB	
1546	Belgium Blue	Polled	BB	
1552	Belgium Blue	Polled	BB	
1554	Belgium Blue	Polled	BB	
1560	Belgium Blue	Polled	BB	
1564	Belgium Blue	Polled	BB	
1573	Belgium Blue	Polled	BB	
1574	Belgium Blue	Polled	BB	
1582	Belgium Blue	Polled	BB	
1585	Belgium Blue	Polled	BB	
1586	Belgium Blue	Polled	BB	
1525	Belgium Blue	Polled	BB	

A.3 Shorthorn Raw Data

Table A4: Shorthorn Raw Data, from Farm F (n=74)

Tag	Breed	Phenotype	PC region	Mismatch
1	Shorthorn	Horned	AA	
3	Shorthorn	Horned	AA	
4	Short Horn	Horned	AA	
5	Short Horn	Horned	AA	
6	Short Horn	Horned	AA	
8	Short Horn	Horned	AA	
9	Short Horn	Horned	AA	
10	Short Horn	Horned	AA	
11	Short Horn	Horned	AA	
12	Short Horn	Horned	AA	
15	Short Horn	Horned	AA	
18	Short Horn	Horned	AA	
19	Short Horn	Horned	AA	
20	Short Horn	Horned	AA	
21	Short Horn	Horned	AA	
22	Short Horn	Horned	AA	
23	Short Horn	Horned	AA	
32	Short Horn	Horned	AA	
33	Short Horn	Horned	AA	
45	Short Horn	Horned	AA	
46	Short Horn	Horned	AA	
47	Short Horn	Horned	AA	
48	Short Horn	Horned	AA	
54	Short Horn	Horned	AA	
55	Short Horn	Horned	AA	
57	Short Horn	Horned	AA	
59	Short Horn	Horned	AA	
65	Short Horn	Horned	AA	
70	Short Horn	Horned	AA	
73	Short Horn	Horned	AA	
76	Short Horn	Horned	AA	
78	Short Horn	Horned	AA	
80	Short Horn	Horned	AA	
81	Short Horn	Horned	AA	
83	Short Horn	Horned	AA	
91	Short Horn	Horned	AA	
93	Short Horn	Horned	AA	
96	Short Horn	Horned	AA	
97	Short Horn	Horned	AA	
99	Short Horn	Horned	AA	
101	Short Horn	Horned	AA	
104	Short Horn	Horned	AA	

14	Short Horn	Horned	AA	
17	Short Horn	Horned	AA	
40	Short Horn	Horned	AA	
84	Short Horn	Horned	AA	
89	Short Horn	Horned	AA	
67	Short Horn	Horned	AB	*
66	Short Horn	Polled	AA	*
7	Short Horn	Polled	AB	
13	Short Horn	Polled	AB	
27	Short Horn	Polled	AB	
28	Short Horn	Polled	AB	
29	Short Horn	Polled	AB	
30	Short Horn	Polled	AB	
36	Short Horn	Polled	AB	
37	Short Horn	Polled	AB	
39	Short Horn	Polled	AB	
43	Short Horn	Polled	AB	
52	Short Horn	Polled	AB	
53	Short Horn	Polled	AB	
56	Short Horn	Polled	AB	
60	Short Horn	Polled	AB	
63	Short Horn	polled	AB	
71	Short Horn	Polled	AB	
74	Short Horn	Polled	AB	
77	Short Horn	Polled	AB	
79	Short Horn	Polled	AB	
85	Short Horn	Polled	AB	
98	Short Horn	Polled	AB	
16	Short Horn	Polled	BB	
41	Short Horn	Polled	BB	
58	Short Horn	Polled	BB	
100	Short Horn	Polled	BB	

A.4 Hereford Raw Data

Table A5: Hereford Raw Data, from Farm G (n=44)

Tag	Breed	Phenotype	PC region	Mismatch
0065160047	Hereford	Polled	AA	*
0065160071	Hereford	Polled	AA	*
0065160001	Hereford	Polled	AB	
0065160004	Hereford	Polled	AB	
0065160005	Hereford	Polled	AB	
0065160010	Hereford	Polled	AB	
0065160014	Hereford	Polled	AB	
0065160023	Hereford	Polled	AB	
0065160025	Hereford	Polled	AB	
0065160030	Hereford	Polled	AB	
0065160032	Hereford	Polled	AB	
0065160045	Hereford	Polled	AB	
0065160053	Hereford	Polled	AB	
0065160054	Hereford	Polled	AB	
0065160059	Hereford	Polled	AB	
0065160067	Hereford	Polled	AB	
0065160070	Hereford	Polled	AB	
0065160079	Hereford	Polled	AB	
0065160081	Hereford	Polled	AB	
0065160083	Hereford	Polled	AB	
0065160084	Hereford	Polled	AB	
0065160086	Hereford	Polled	AB	
0065160087	Hereford	Polled	AB	
0065160090	Hereford	Polled	AB	
0065160003	Hereford	Polled	BB	
0065160008	Hereford	Polled	BB	
0065160009	Hereford	Polled	BB	
0065160011	Hereford	Polled	BB	
0065160012	Hereford	Polled	BB	
0065160013	Hereford	Polled	BB	
0065160015	Hereford	Polled	BB	
0065160029	Hereford	Polled	BB	
0065160034	Hereford	Polled	BB	
0065160035	Hereford	Polled	BB	
0065160048	Hereford	Polled	BB	
0065160051	Hereford	Polled	BB	
0065160055	Hereford	Polled	BB	
0065160057	Hereford	Polled	BB	
0065160058	Hereford	Polled	BB	
0065160060	Hereford	Polled	BB	
0065160072	Hereford	Polled	BB	
0065160077	Hereford	Polled	BB	

0065160089	Hereford	Polled	BB	
0065160095	Hereford	Polled	BB	

Table A6: Hereford Raw Data, from Farm D (n=90)

Tag	Breed	Phenotype	PC region	Mismatch
1430020033	Hereford	Scurred	AA	
1430060017	Hereford	Pure Horned	AA	
1430140107	Hereford	Pure Horned	AA	
1430150007	Hereford	Pure Horned	AA	
1430060052	Hereford	Pure Horned	AA	
1430150010	Hereford	Pure Horned	AA	
1430150012	Hereford	Pure Horned	AA	
1430150016	Hereford	Pure Horned	AA	
1430150017	Hereford	Pure Horned	AA	
1430150018	Hereford	Polled	AA	*
1430150019	Hereford	Pure Horned	AA	
1430150023	Hereford	Pure Horned	AA	
1430150030	Hereford	Horned	AA	
1430070006	Hereford	Pure Horned	AA	
1430150035	Hereford	Horned	AA	
1430150046	Hereford	Horned	AA	
1430150056	Hereford	Pure Horned	AA	
1430150058	Hereford	Pure Horned	AA	
1430150067	Hereford	Pure Horned	AA	
1430150076	Hereford	Polled	AA	*
1430150082	Hereford	Pure Horned	AA	
1430150105	Hereford	Pure Horned	AA	
1430150083	Hereford	Pure Horned	AA	
1430070041	Hereford	Pure Horned	AA	
1430070043	Hereford	Polled	AA	*
1430070057	Hereford	Pure Horned	AA	
1430070066	Hereford	Polled	AA	*
1430070088	Hereford	Pure Horned	AA	
1430030056	Hereford	Pure Horned	AA	
1430080044	Hereford	Pure Horned	AA	
1430080046	Hereford	Pure Horned	AA	
1430090020	Hereford	Pure Horned	AA	
1430090022	Hereford	Horned	AA	
1430090029	Hereford	Pure Horned	AA	
1430090034	Hereford	Pure Horned	AA	
1430090035	Hereford	Pure Horned	AA	
1430090040	Hereford	Pure Horned	AA	
1430090043	Hereford	Polled	AA	*
1430090074	Hereford	Pure Horned	AA	
1430100018	Hereford	Pure Horned	AA	

1430100023	Hereford	Horned	AA	
1430100057	Hereford	Horned	AA	
1430100088	Hereford	Pure Horned	AA	
1430110009	Hereford	Pure Horned	AA	
1430110041	Hereford	Pure Horned	AA	
1430110044	Hereford	Pure Horned	AA	
1430110086	Hereford	Pure Horned	AA	
1430110087	Hereford	Horned	AA	
1430110099	Hereford	Pure Horned	AA	
1430110101	Hereford	Pure Horned	AA	
1430110109	Hereford	Pure Horned	AA	
1430120011	Hereford	Pure Horned	AA	
1430120015	Hereford	Pure Horned	AA	
1430120017	Hereford	Horned	AA	
1430120021	Hereford	Pure Horned	AA	
1430120037	Hereford	Horned	AA	
1430120042	Hereford	Pure Horned	AA	
1430120043	Hereford	Horned	AA	
1430120062	Hereford	Polled	AA	*
1430050012	Hereford	Pure Horned	AA	
1430130001	Hereford	Polled	AA	*
1430130052	Hereford	Pure Horned	AA	
1430130054	Hereford	Pure Horned	AA	
1430130056	Hereford	Pure Horned	AA	
1430130103	Hereford	Pure Horned	AA	
1430140010	Hereford	Pure Horned	AA	
1430140021	Hereford	Pure Horned	AA	
1430140042	Hereford	Pure Horned	AA	
1430140057	Hereford	Horned	AA	
1430140068	Hereford	Pure Horned	AA	
1430140088	Hereford	Polled	AB	
1430150008	Hereford	Polled	AB	
1430150011	Hereford	Polled	AB	
1430150033	Hereford	Polled	AB	
1430090017	Hereford	Polled	AB	
1430090027	Hereford	Polled	AB	
1430040003	Hereford	Polled	AB	
1430100013	Hereford	Polled	AB	
1430110048	Hereford	Polled	AB	
1430110064	Hereford	Polled	AB	
1430110078	Hereford	Polled	AB	
1430120056	Hereford	Polled	AB	
1430120067	Hereford	Polled	AB	
1430120072	Hereford	Polled	AB	
1430130025	Hereford	Polled	AB	
1430130035	Hereford	Polled	AB	
1430130092	Hereford	Horned	AB	*

0494070709	Hereford	Polled	AB	
1430100070	Hereford	Polled	BB	
1430130023	Hereford	Polled	BB	

Table A7: Hereford Raw Data, from Farm E (n=26)

Tag	Breed	Phenotype	PC region	Mismatch
SB2	Hereford	Horned	AA	
SB3	Hereford	Horned	AA	
1532060074	Hereford	Pure Horned	AA	
1177060155	Hereford	Pure Horned	AA	
1177060170	Hereford	Pure Horned	AA	
1177060173	Hereford	Pure Horned	AA	
51050531	Hereford	Polled	AA	*
	Hereford	Polled	AA	*
51060610	Hereford	Polled	AA	*
51070719	Hereford	Polled	AA	*
51070772	Hereford	Polled	AA	*
	Hereford	Polled	AB	
51050505	Hereford	Polled	AB	
51060676	Hereford	Polled	AB	
51070713	Hereford	Polled	AB	
51070726	Hereford	Polled	AB	
51070770	Hereford	Polled	AB	
51070793	Hereford	Polled	AB	
SB4	Hereford	Horned	BB	*
51060608	Hereford	Polled	BB	
51060687	Hereford	Polled	BB	
51070705	Hereford	Polled	BB	
51070753	Hereford	Polled	BB	
51070755	Hereford	Polled	BB	
51070798	Hereford	Polled	BB	
51070811	Hereford	Polled	BB	

A.5 Holstein-Friesian Raw Data

Table A8: Holstein-Friesian and Holstein-Friesian Hereford Cross Raw data, from Farm H (n=4)

Tag	Breed	Phenotype	PC region	Mismatch
131	Holstein Friesian	Horned	AA	
183	Holstein Friesian	Horned	AA	
150	Holstein Friesian/cross	Polled	AB	
162	Holstein Friesian/cross	Polled	AB	

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